# STUDIES ON MAGNESIUM METABOLISM IN RUMINANTS

by

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## GENERAL INTRODUCTION

Tetany characterised by a low level of serum magnesium is an important cause of economic loss in lactating ruminants. Although we understand little of the factors controlling the concentration of magnesium in the serum of ruminants it appears to be well established that the maintenance of normal levels soon fails when dietary intake becomes inadequate for the animal's requirements. For example, starvation can lead to a rapid fall in serum magnesium in lactating animals over periods as short as 5 days (Inglis, Weipers and Pearce, 1959; Robertson, Paver, Barden and Marr, 1960). Conversely, supplementation of the diet with inorganic salts of magnesium can reduce the incidence of tetany in cattle (Blakemore and Stewart, 1934-5; Allcroft, 1947; Allcroft, 1953; Stewart, 1954). Various authors (e.g. Blaxter and McGill, 1956; Rook and Balch, 1958) have advanced theories consistent with these basic facts to account for the increased incidence and rapid onset of hypomagnesaemic tetany in lactating animals at the beginning of spring grazing. Present knowledge, however, is inadequate to test them in detail since information is lacking on the processes of absorption and excretion of the element, which would allow the expression of requirements in terms of dietary magnesium.

### Absorption

The rumen is the main site for the liberation of magnesium from the plant cell. Garner (1949) effected the liberation of magnesium from sterile grass powder by the incubation *in vitro* under conditions simulating

those of the rumen. At pH 6.9 some 80 per cent of magnesium was recovered in the ultrafilterable form after 36-72 hours. A slight increase in this value to some 85 per cent occurred when ruminal organisms were present. By in vitro incubation in saliva Garton (1951) demonstrated that the mechanism is a simple solution of the ion from the food. Both authors agree that a further small increment is probably released in the abomasum, since Garner's figure was increased by a further 10 per cent when the pH was reduced to 2, and Garton obtained an increased liberation by incubating ruminal contents for 3 hours with gastric juice.

Stewart and Moodie (1956) demonstrated absorption at all sections of the alimentary tract from rumen to caecum when the magnesium concentration in the rumen was raised to abnormally high levels by oral dosing or local injection of inorganic magnesium salts. They suggested that the principal site of magnesium absorption is the duodenum and small intestine. Smith (1959a) found that from 25 per cent to 40 per cent of dietary magnesium is absorbed by the large intestine of milk fed calves (2 to 4 weeks) but by the age of 6 to 12 weeks the large intestine ceased to have this function (Smith, 1959b).

Little is known regarding the availability of magnesium in feeding-stuffs and supplements, and there is confusion in the literature as to the meaning of the term. In this study availability is defined as the percentage absorption of dietary magnesium. Its determination is complicated by the fact that the magnesium in the faeces originates not only from the diet but also from digestive secretions.

Two general techniques using radioactive tracers have been developed for measuring these two faecal fractions; the comparative-balance method

of Hansard, Comar and Plumlee (1954) and the isotope-dilution technique of Visek, Monroe, Swanson and Comar (1953). Recently both these methods have been adapted for $^{28}Mg$, the former by Field (1959) and the latter by MacDonald, Care and Nolan (1959). The values obtained for the availability of magnesium in hay to wethers with the two techniques were in good agreement, but in both cases the figure of approximately 26 per cent refers only to a single animal.

Field, McCallum and Butler (1958) showed that the relationship between urinary and dietary magnesium can be used to determine availability provided the endogenous faecal excretion remains constant. Using this technique they found that for two wethers spring herbage samples had magnesium availabilities of 13 per cent and 26 per cent irrespective of whether they came from fields where hypomagnesaemic tetany had or had not occurred. Rook, Balch and Line (1958) and Rook and Balch (1958), ignoring endogenous faecal excretion, have used the digestion coefficient, referred to by them as "availability", as a comparative measure of the utilization of the magnesium in a variety of diets by cows. Their values showed a wide variation between individual cows on the same diet (23 per cent to 34 per cent) and a range of 10 per cent to 40 per cent for the mean digestion coefficient of the magnesium in different typical winter rations. When the diet of their cows was changed suddenly from winter rations to cut spring herbage, involving a considerable reduction in intake, the digestion coefficient of the magnesium in spring herbage was 20 per cent, which they concluded was unusually low.

The availability of milk magnesium for calves falls rapidly with age.

Values of 70 per cent to 90 per cent have been reported for very young calves (2 to 4 weeks) (Smith, 1957) and only 30 per cent to 50 per cent for older calves (Blaxter and Rook, 1954; Smith, 1959c).

If the amount of magnesium excreted in the urine is taken as an index of absorption, a number of soluble magnesium salts (citrate (Bogert and McKittrick, 1922), lactate (Carswell and Winter, 1931), chloride (Taylor and Winter, 1929), and sulphate (Hart and Steenbock, 1913)) would seem to be readily absorbed. Stewart and Moodie (1956) showed that magnesium was much more quickly and efficiently absorbed after doses of magnesium nitrate by mouth than after similar amounts of magnesium sulphate.

There is no direct evidence on whether the absorption of magnesium is an active or a passive process. Koefoed-Johnsen and Ussing (1960) have defined a passive process as one which can be accounted for by means of ordinary physical forces, including concentration gradients, electric gradients, solvent drag or any combination of these. Active transport processes are those which involve the participation of some energy-yielding chemical reaction.

Absorption does not appear to depend upon the presence of an acid or basic reaction in the intestine, since addition of 2 per cent of sodium carbonate to the diet of rats did not affect utilization (Forbes and Pitts, 1935) nor did addition of hydrochloric acid to the diet of dogs affect either the absorption or the partition between urine and faeces (Givens, 1918a).

The addition of milk to the diet appears to have a beneficial effect on magnesium absorption. Givens (1918a) found that the addition of raw or

dried milk to the normal diets of adult human subjects increased the urinary excretion of magnesium. With dogs the addition of dried skimmed milk changed negative to positive magnesium balances and the effect was greater than could be accounted for by the increment of magnesium in the added milk. When hydrochloric acid was added with the milk the balance became negative (Givens and Mendel, 1917).

Head and Rook (1955) administered large doses of ammonium salts directly into the rumen of dairy cows and found that this was followed by a reduction in urinary magnesium excretion. They interpreted this result as due to a reduction in magnesium absorption possibly by the formation of insoluble magnesium ammonium phosphate. Blaxter and McGill (1956) and Wilson (1960), however, have stressed the need for caution when interpreting changes in urinary magnesium as indicative of changes in intestinal absorption.

From their studies on the absorption of magnesium from the alimentary tract of sheep, Stewart and Moodie (1956) could find no evidence to show that calcium and magnesium follow a similar pathway of metabolism. On the other hand, Alcock and MacIntyre (1960) postulated the existence of a common transport mechanism for magnesium and calcium ions in the intestine of rats. Their results showed that the two elements competed for absorption. For instance, in rats on a calcium deficient diet magnesium absorption was increased and the increase was equivalent to the calcium absorbed by rats on normal diets. These results are not in agreement with those of Lengemann (1959) who found that the percentage absorption of magnesium increased with increasing proportions of calcium to magnesium in the diet.

Evidence for active transport of magnesium across the gut wall is found in a recent observation by Graham, Caeser and Burgen (1960) that the percentage absorption of $^{28}Mg$ increased with decreasing magnesium intake.

Meintzer and Steenbock (1955) studied the effect of vitamin D on the absorption of magnesium by rats on a diet low in calcium and phosphorus. The addition of vitamin D increased the absorption of magnesium given as the carbonate, phosphate and phytate. In contrast, the absorption of magnesium from the milk by calves was not affected by the addition of vitamin D to the diet nor by the irradiation of the calves with ultra violet light (Smith, 1958).

## Excretion

The major systems responsible for the excretion of magnesium are the gastro-intestinal tract, the kidney, and the mammary gland during lactation, with the skin and its appendages playing a minor part.

## Gastro-Intestinal Tract

The main route of magnesium loss is *via* the faeces. The results of Nicolaysen (1936) indicated that the magnesium, like the calcium of faeces, is unabsorbed dietary magnesium with a small amount derived from intestinal secretions. Studies with $^{28}Mg$ have confirmed this viewpoint, since, after parenteral administration, $^{28}Mg$ is recoverable from the faeces of sheep, dog, man and rat. In other words, the gastro-intestinal tract acts as a true excretory organ, but its role in this respect varies with the species. Zumoff, Bernstein, Imarisis and Hellman (1958) and Silver,

Robertson and Dahl (1960) found in man that less than 1 per cent of the parenterally administered dose of $^{28}Mg$ is excreted in the faeces, whereas in sheep (Field, 1959; MacDonald et al., 1959) about 20 per cent is excreted by this route.

Magnesium re-enters the gastro-intestinal tract in the digestive secretions, of which the saliva and gastric juice appear to be the most important on account of their large volume (Table 1).

Table 1

Magnesium content of digestive secretions

| Digestive secretion | Vol. (l/day) | Conc. of Mg (mg/100ml) | Output of Mg (mg/day) |
|---|---|---|---|
| Saliva | 4 - 16[4] | 0.6 - 1.0[3] | 36 - 160 |
| Gastric juice | 4 - 5[1] | 0.4[5]<br>0.7[2] | 16 - 20<br>28 - 35 |
| Bile | 0.5 - 0.6[1] | 1.3[5] | 6 - 8 |
| Pancreatic juice | 0.3[1] | 0.8[5] | 2.5 |
| | | Total | 60 - 200 |

1. Hill and Harrison (1960)
2. Garton (1951)
3. McDougall (1948)
4. Kay (1960)
5. Field (1960b)

Table 1 shows that the total daily secretion into the gastro-intestinal tract of sheep lies between 60 and 200 mg, and this is of the same order as the values found for the endogenous faecal loss by this species (Field et al., 1958; Field, 1959; MacDonald et al., 1959).

By definition endogenous magnesium excretion is the faecal loss occurring while the subject is on a magnesium-free diet or starved. Unfortunately, there is no practical method for its direct estimation, since the formulation of palatable magnesium-free rations for ruminants is as yet impossible, and, alternatively, the period of starvation required would involve cruelty. This has led to the use of indirect methods, which are the same as those used for determining availability, since once either availability or endogenous faecal excretion is determined the other may easily be derived.

Blaxter and Rook (1954) used the relationship between magnesium retention and magnesium intake to calculate the total endogenous loss of magnesium in milk-fed calves. This value (3-4 mg/Kg body wt./day) may be taken as the endogenous faecal loss since the loss via the urine is negligible (Smith, 1957). Smith (1959c) found the endogenous faecal loss of calves to be about 0.5 mg/Kg body wt./day at 2 to 5 weeks of age and 2.2 mg/Kg body wt./day at 26 to 32 weeks of age. Blaxter and McGill (1956) have reported a figure of 3 to 5 mg/Kg body wt./day for cows, but no experimental details were given.

Using the relationship between urinary excretion and dietary intake of magnesium to determine endogenous faecal magnesium, Field et al., (1958) obtained values of 1.7 and 5.0 mg/Kg body wt./day for two adult wethers.

Using $^{28}Mg$, Field (1959) and MacDonald et al. (1959) found values of 4.5 and 5.0 mg/Kg body wt./day respectively for adult wethers.

There is no direct evidence on the relationship between absorption and endogenous faecal excretion, nor on whether an animal whose serum magnesium level is below the normal range can conserve body magnesium by reducing endogenous faecal loss.

Kidney

The kidney plays an important role in the regulation of the concentration of magnesium in the extra-cellular fluid. It prevents the concentration from rising too high by excreting what is absorbed in excess of the body's requirements. Comparatively little is known, however, about the mechanisms involved.

Wilson (1960) has critically reviewed existing knowledge on renal magnesium excretion and concluded with Barker, Elkinton and Clarke (1959) that excretion of magnesium is by a filtration-reabsorption mechanism, but admits that the question of kidney mechanism really remains open. Recently Murdaugh and Robinson (1960) have shown that tubular reabsorption is an active process and takes place in the first portion of the distal nephron.

The basic equation for a filtration-reabsorption mechanism is

$$U_{Mg} V = P_{Mg} F - T_{Mg}$$

where $U_{Mg}$ is the concentration of magnesium in the urine, V the volume of urine, F the glomerular filtration rate, $P_{Mg}$ the concentration of ultrafilterable magnesium in the plasma and $T_{Mg}$ the maximum tubular reabsorption

rate. From this equation it follows that, if the tubular reabsorption rate is constant, magnesium is a threshold substance and urinary magnesium excretion is linearly related to the concentration of ultra-filterable magnesium in the plasma.

The relationship between ultra-filterable plasma magnesium and urinary magnesium has not been studied in the ruminant subject. Rook et al. (1958) found a rectilinear relationship between serum or plasma magnesium concentration and the urinary magnesium content of cows and estimated the renal threshold for serum magnesium to be not greater than 2.15 mg/ 100 ml. In contrast, Field et al. (1958) could find no evidence for such a relationship with sheep. Wilson (1960) reported values of 2 mg/ min. and 100 ml./min. for $^{T}Mg$ and F in ewes and he calculated the renal threshold concentration in plasma to be about 2 mg/100 ml.

Changes in urinary magnesium excretion can result from impaired renal function, due either to a changed glomerular filtration rate or to a changed tubular reabsorption rate. Robinson, Murdaugh and Pexhel (1959) have shown that in human cases of glomerular damage urinary magnesium excretion is reduced and that patients develop hypermagnesaemia when the filtration rate falls below 30 ml./min. Hammarsten, Allgood and Smith (1957) observed a 60 $\pm$ 14.7 per cent tubular reabsorption of magnesium in patients with renal disease while in normals it was 94 $\pm$ 1 per cent. Changes in tubular reabsorption may arise from the fact that reabsorbed fluid must contain equivalent amounts of cations and anions. This is responsible for the increased excretion of magnesium in experimental acidosis produced by ingestion of ammonium chloride (Tibbetts and Aub, 1937; Jabir, Roberts and Womersley, 1957).

In a recent investigation of the relationship between the transport system of magnesium and calcium, Samiy, Brown, Globus, Kessler and Thompson (1960) found that the excretion of these elements is reciprocally modified, probably indicating competition for a common reabsorption mechanism. This confirms the results of earlier work, namely, that injection of calcium salts increases the renal excretion of magnesium (Mendel and Benedict, 1909; Greenwald and Gross, 1925).

McCance and Widdowson (1942) found that urinary magnesium excretion is correlated with apparent absorption of magnesium in man. On the other hand, Field et al. (1958) could find no evidence for such a relationship with sheep, but found that urinary magnesium was rectilinearly related to dietary magnesium.

The increased incidence of hypomagnesaemic tetany in dairy cows at the onset of grazing in the spring has led a number of workers, including Rook and Balch (1958), Field et al. (1958), to investigate the effect of abrupt changes in diet on the urinary magnesium excretion of ruminants. Rook and Balch (1958) investigated the effect on the urinary magnesium excretion of dairy cows of a change from typical winter rations to cut spring herbage involving a marked reduction in magnesium intake. The spring herbage, cut daily from the same pasture at two stages of growth, was offered to the cows in excess of appetite. After a change to the less mature herbage they found an immediate fall in urinary magnesium and interpreted it as evidence of reduced intestinal absorption due to low magnesium content and availability. With the more mature spring herbage they observed a rise in urinary magnesium after an initial fall and

tentatively attributed it to increased magnesium intake consequent upon a higher dry matter consumption occurring after the first 2 days. For a dietary change from one to another spring herbage, Field et al. (1958) found that the urinary magnesium excretion of sheep quickly reflected differences in magnesium intakes between rations.

Mammary Gland

Magnesium is a major mineral constituent of milk, with concentrations of 4, 12, 16 and 31 mg./100 ml. in human, cow, goat and rat whole milk respectively. Only about 20 per cent of the magnesium in cow's milk is in the ionic form (Van Kreveld and Van Minnen, 1955), some 30 per cent is associated with the colloid, and the remaining 50 per cent in forms unknown (Alexander and Ford, 1957).

Milk is a major route of excretion of magnesium from the body; loss by this route in a heavily lactating dairy cow may amount to 3 g. per day, which represents a large proportion of the dietary magnesium absorbed from the gut by these animals. Any factor affecting either the rate of secretion or the concentration of magnesium in milk will in turn modify the requirements of magnesium for milk production. The factors affecting the rate of secretion are well documented and will not be referred to here. As regards variation in magnesium concentration, the reports are so few that no conclusions can be drawn.

## OUTLINE OF RESEARCH PROGRAMME

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From this résumé of the literature on absorption and excretion it is apparent that there is very little qualitative and quantitative information on many important aspects of these subjects. The recent introduction of a radioactive isotope of magnesium, $^{28}Mg$, provided an opportunity to study the dynamics of absorption and secretion in the gastro-intestinal tract. Consequently, it was decided to study in Section I of this thesis the distribution of $^{28}Mg$ in the ingesta and mucosa of the gut of sheep 10 h. after oral or intravenous administration of single doses. The objective of the experiment was twofold; first, to obtain information on the sites of absorption and secretion of magnesium, and, secondly, to investigate the validity of some of the assumptions inherent in the use of $^{28}Mg$ for the determination of availability and endogenous faecal magnesium excretion. In addition it was possible to measure the rate at which $^{28}Mg$ in the plasma exchanged with the stable magnesium in the tissues.

To see whether the results obtained in the above experiment for the distribution of magnesium along the gastro-intestinal tract were independent of the interval between the last feed and the killing of the sheep, the diurnal variation in the concentration of magnesium in the faeces of sheep was investigated. Moore and Tyler (1955a) observed a marked diurnal variation in the concentration of calcium and phosphorus in the faeces of pigs. They found that these elements passed along the tract more

rapidly than the dry matter and that hence the distribution of calcium and phosphorus in the tract was dependent upon the time between the last meal and the killing of the pig.

Because of the importance of ascertaining the cause of the increased incidence of hypomagnesaemic tetany in dairy cows at the onset of grazing in the spring, it was decided to study in Section II the effect of abrupt changes in the nature of the diet on the urinary magnesium excretion of sheep.

In the experiments reported by Rook and Balch (1958), a change from typical winter rations to cut spring herbage produced an immediate fall in the urinary magnesium excretion of dairy cows, which was interpreted as evidence of a reduced intestinal absorption due to the lower content and availability of magnesium in spring herbage. These experiments did not reveal how much the reduced intake of magnesium contributed to the decrease in urinary magnesium excreted, and this particular aspect has received special emphasis in the experiments described in Section II.

Field et al. (1958) reported a series of balance trials in which sheep were given spring herbage collected from a variety of pastures. It was found that there was a rectilinear relationship between urinary magnesium excretion and dietary magnesium intake and that the regression coefficient varied considerably from sheep to sheep. From these data tentative figures were calculated for the availability of dietary magnesium.

In view of the importance of these findings in relation to the aetiology of hypomagnesaemic tetany in sheep and the development of methods for the determination of availability, it was decided to confirm and extend

these observations. A series of balance trials were carried out in which each of four wethers was given three different amounts of the same sample of grass nuts for successive 15 day periods. The balance trials were repeated at an interval of one year. These experiments, described in Section III, have been extended to include calcium because of the known physiological interaction between the two elements.

# DEFINITIONS AND GENERAL METHODS

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The following terms, methods and equipment are referred to in this thesis.

### Metabolism crate

The crate (Plate 1) was similar to the one recently described by MacDonald (1958). It was made of wood and accommodated two sheep, the two being separated by a partition, the upper half of which was made of glass reinforced with small wire mesh and the lower half of double hardboard. The floor was made of wood and covered with a layer of $\frac{1}{8}$ in. rubber sheeting, which was removeable for easy cleaning. The feeding box was completely enclosed and lined with aluminium. The top opened for feeding and the lower half of the front for the removal of food residues (Plate 2). Polythene water troughs were fixed on the outside of the crate.

### Faecal collection

The harness for the collection of faeces has been described by MacDonald (1958) and is shown in Plate 3.

The faeces were collected in a Polythene bag, of known weight. At the end of a 24 h. period the bag and contents were weighed and the weight of faeces calculated by difference. The contents were mixed and a representative sample was taken for chemical and dry matter determination. This procedure obviated the need for the quantitative removal of the faeces from the Polythene bag, a difficult operation when the faeces were soft.

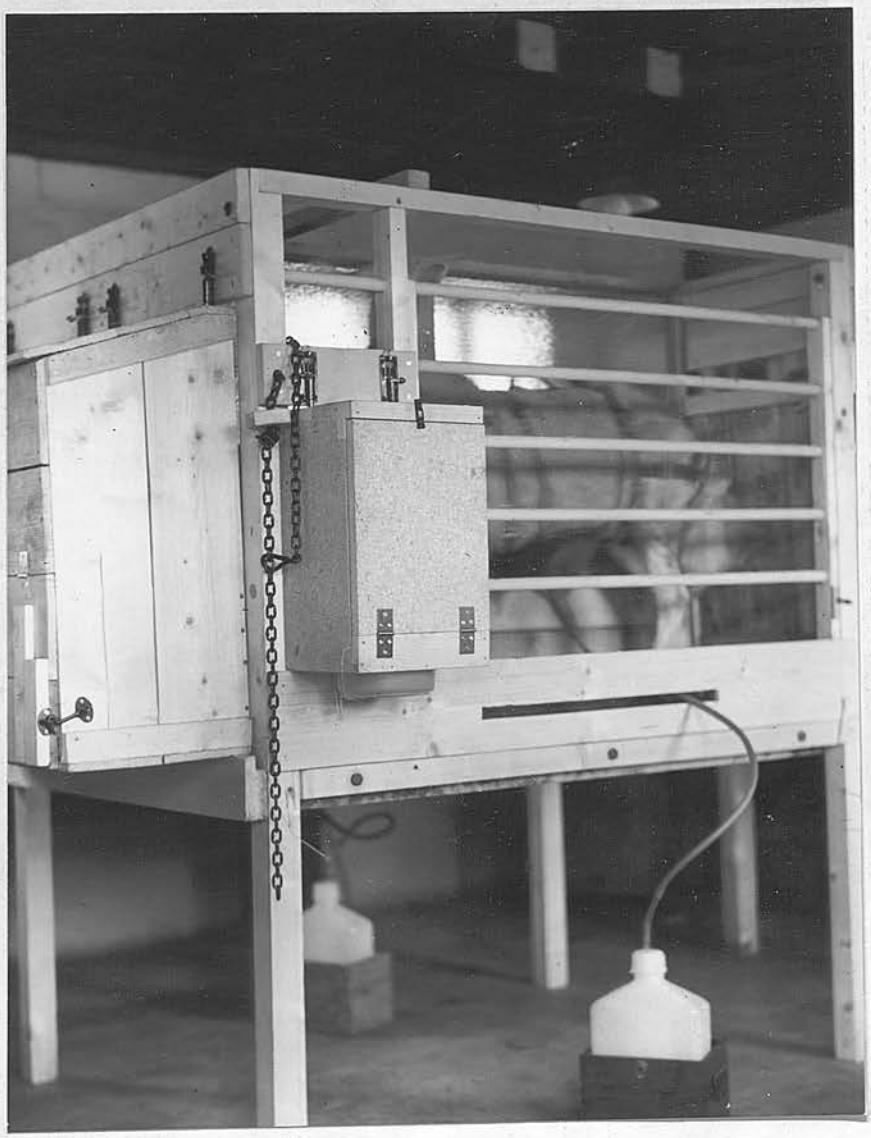


Plate 1. Metabolism Crate

Plate 2. Feeding Box

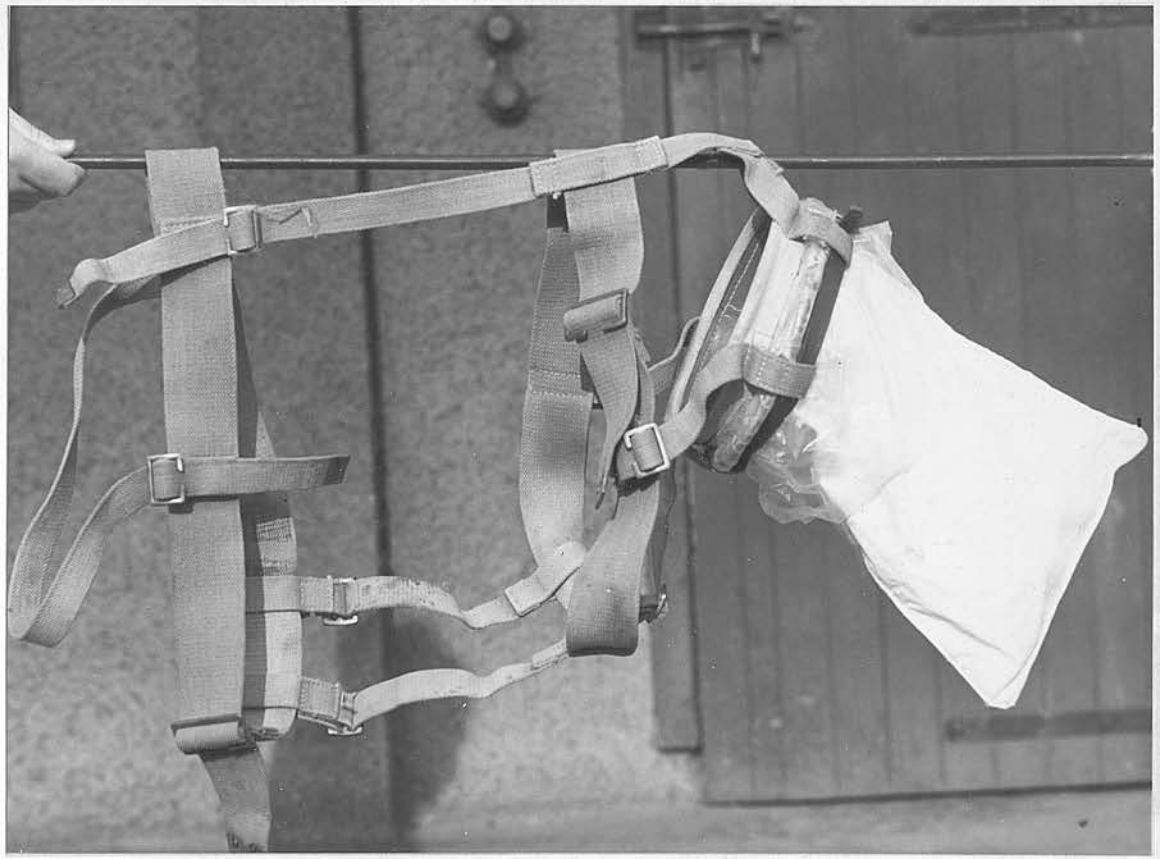


Plate 3. Harness for Collection of Faeces

**Plate 4. Urine Funnel**

Urine collection

The urine was collected in a rubber funnel made by the Avon Rubber Company, Bristol, according to the design of Raymond, Harris and Harker (1953). The tube from the funnel passed through a slit in the side of the crate and into a 5 l. Polythene receiver containing acetic acid (Plate 4).

Dry matter

The sample was well mixed and about 300-500 g. of spring herbage, hay, grass nuts or faeces was transferred to a metal container and weighed accurately. For the spring herbage, the sides and bottom of the container were perforated for better air circulation. The container was then placed in a convection oven set at 105° and dried to a constant weight. This usually took 48 h.

Preparation of sample

The dried sample was ground in a Christy Norris mill with a 1 mm. sieve and stored in a glass bottle with a screw top. All aliquots removed from the bottle for chemical estimation were corrected for the moisture gained during storage.

Ashing

For the determination of calcium and magnesium, samples of herbage, grass nuts, faeces or urine were wet ashed by the procedure of Middleton and Stuckey (1954).

Transfer about 1 g. of the solid samples or 100 ml. urine to a 600 or

800 ml. Pyrex or Vitreosil beaker and add 10 ml. of concentrated $HNO_3$ containing 5% concentrated $H_2SO_4$. Add 10 ml. of water to the solid samples to prevent bumping. Heat the mixture on a hot plate at 250° until dry, cool, add a further 5 ml. concentrated $HNO_3$ and reheat until dry. Repeat the process until the residue is white with no specks of carbon. Dissolve the residue by heating for 30 mins. first with 10 ml. 5% (w/v) NaOH and then with 25 ml. of approximately N-HCl. At the NaOH stage, remove residue adhering to the inside of the beaker by scratching with a glass rod. Interfering elements such as iron, aluminium and manganese are then removed by the method recommended by Davidson (1952). This consists of buffering the ash solution to pH 5.0 with sodium acetate and treating with bromine water to precipitate manganese as the hydrated oxide. At this pH, iron and aluminium are also precipitated. Add 5 ml. 50% (w/v) sodium acetate to the ash solution and adjust the pH to about 5.0 with NaOH using bromophenol blue as indicator. Add 0.5 ml. of saturated solution of liquid bromine in distilled water and heat until the solution is colourless. Filter into a 50 or 100 ml. volumetric flask and wash beaker thoroughly with water. After cooling the filtrate and washings make up to volume.

Determination of calcium in ash solution and serum

Calcium was determined throughout by a modification of the method described for the EEL flame photometer by Powell (1953).

Pipette 1 ml. of the ashed sample solution or 1.5 ml. of serum into a centrifuge tube and add 4 ml. of water and 1 ml. of saturated ammonium

oxalate solution. Mix well and allow to stand at room temperature for 30 mins.

Centrifuge at 2-3000 r.p.m. for 15 mins. and decant the supernatant fluid. Wash the precipitate with 3 ml. of 2% ammonia, re-centrifuge and dissolve in 5 ml. of 0.05N perchloric acid. Read in the flame photometer setting the galvanometer at 50 with a solution containing 40 mg. Ca/l.

If the reading for the sample is below 15 or above 60 repeat the determination with a suitable aliquot making the volume up to 5 ml. with water.

Determination of magnesium in ash solution

Magnesium was estimated with 8-hydroxyquinoline by the Butler and Field (1956) modification of the method of Davidson (1952).

Add to 15 ml. conical centrifuge tubes, by means of a pipette, up to 4 ml. amounts of solution containing 20 to 200 µg of magnesium and make up to 4 ml. with distilled water. Prepare reagent blanks with 4 ml. of distilled water. Add 0.5 ml. of 0.1N ammonium oxalate and rotate the tubes to mix the solution. Place the centrifuge tubes in a water bath at 100° for 30 mins. and then leave them overnight to precipitate the calcium oxalate. Centrifuge at 3000 r.p.m. for 15 mins. and decant the supernatant into another set of 15 ml. Pyrex centrifuge tubes. Wash precipitate with 2 ml. of distilled water, centrifuge for 15 mins. and add the washings to the supernatant fluids. Add exactly 0.5 ml. of a freshly prepared 0.5% (w/v) solution of 8-hydroxyquinoline in 2N acetic acid to the combined supernatant liquors and washings and mix by rotation. Add 3 ml. of ammonium hydroxide sp.gr. 0.880, mix, and place in a water bath at 60° for

90 mins. Allow to stand at room temperature for at least 60 mins. and then centrifuge at 3000 r.p.m. for 15 mins. Withdraw the supernatant liquor to within 1 mm. of the surface of the precititate by means of a suction bottle and tube with its tip drawn out and bent. Re-suspend the precipitate by sharply tapping the end of the tube, add 10 ml. of wash solution (a 1%(v/v) concentrated ammonia solution in 96% (v/v) ethyl alcohol) and centrifuge at 3000 r.p.m. for 15 mins. Suck off the supernatant liquor and repeat the washing operation. Dissolve the precipitate in 10 ml. of approximately 0.1N HCl and transfer the solution with the acid to a 25 ml. volumetric flask and make up to volume with acid. With distilled water in the reference cell, measure the optical density of each solution at 358 mμ in 1 cm. cells.

Estimation of magnesium in serum

Serum was analysed for magnesium by the method of Denis (1922) as modified by Hawk, Oser and Summerson (1947).

Precipitate the calcium from 1.5 ml. of serum as in the procedure for calcium in serum. After centrifuging, decant the supernatant liquor into a 10 ml. centrifuge tube. Add 1 ml. concentrated ammonia solution sp.gr. 0.880 and 1 ml. 5% (w/v) solution of ammonium dihydrogen phosphate. Let stand overnight. Centrifuge at 3000 r.p.m. for 30 mins. and pour off the supernatant liquor. Wash precipitate with 5 ml. of 33% (v/v) solution of ammonia, centrifuge for 15 mins. and pour off the wash solution. Repeat the washing a second and third time. Allow the precipitate to stand in a warm place until all the ammonia has evaporated.

Dissolve the precipitate in the tube with 1 ml. 10N $H_2SO_4$ and wash the solution into a 25 ml. volumetric flask. Add 4 ml. molybdate-sulphuric acid reagent (3.75% (w/v) solution of sodium molybdate in 2.5N $H_2SO_4$) and 0.8 ml. of amino napthol sulphonic acid reagent* and make up to 25 ml. with distilled water. Let stand for 2 h. and read on a spectrophotometer at 680 mμ using distilled water as a blank. Colour contributed by reagents is corrected for by a reagent blank containing 1 ml. 10N $H_2SO_4$, 4 ml. molybdate-sulphuric acid reagent and 0.8 ml. of amino napthol sulphonic acid reagent and made up to 25 ml.

To establish the reference curve take 0, 10, 20, 30, 40, 50 and 60 μg of magnesium and treat each solution in the same manner as the sample solution.

*mixture of 2.5 g. amino napthol sulphonic acid, 5 g. sodium sulphite and 146.25 g. sodium metabisulphite dissolved in 1 l.

Determination of $^{28}Mg$

$^{28}Mg$ is an isotope emitting both gamma and beta radiation with a half life of 21.3 h. It decays to $^{28}Al$, which also emits gamma and beta radiation with a half life of 2.3 mins., and this daughter decays to stable $^{28}Si$. The isotope, carrier-free, is produced at the rate of 40 μc per h. in the Harwell cyclotron by the bombardment of KCl with protons at maximum energy. The short half life and the small amounts of $^{28}Mg$ available preclude any treatment of the sample, such as ashing, prior to its estimation. It is therefore measured by its gamma radiation in a scintillation counter.

The samples were placed in a well-type scintillation counter and countered until at least 10,000 counts had been registered; thus giving an accuracy of ± 1 per cent. All counts were corrected for background and decay of the isotope; the latter by counting concurrently with the

unknown samples a small sample of the dose of $^{28}Mg$ given to the sheep. All results are expressed as percentage of the dose x $10^2$.

Specific activity (s.a.)

The ratio of radioactive atoms of an element to total atoms of the same element present in a mixture is called the specific activity of the preparation.

Relative specific activity (r.s.a.)

The ratio of the specific activity of a tissue to that of the blood is the relative specific activity of that tissue.

# SECTION I

## DISTRIBUTION OF $^{28}Mg$ IN THE GASTRO-INTESTINAL TRACT AND TISSUES OF SHEEP

## EXPERIMENTAL

Two 5-year-old wethers received daily (in two equal portions at 10 a.m. and 4 p.m.) 1000 g. of grass nuts containing 1.2 g. Mg. After 6 days on the diet a single dose of 40-60 μc carrier-free $^{28}Mg$, as the chloride, was given at noon by stomach tube to sheep A and by intravenous injection to sheep B. The sheep were killed by exsanguination under nembutal anaesthesia 10 h. later, immediately after sampling of the blood. The abdominal cavity was opened at once and double or single ligatures were tied with as little disturbance of the contents as possible around the junction between the omasum and abomasum, the duodenum immediately adjacent to the pylorus, at numerous points along the small intestine, the ileo-caecal valve, the junction between the caecum and the colon adjacent to the ileo-caecal valve, at two points along the spiral colon and the junction between the distal colon and rectum.

The contents of the gastro-intestinal tract were then removed. Those of the abomasum and sections of the small intestine were weighed, the liquid and solid phases separated by centrifuging at 1400 g. for 20 mins. and the volume of the liquid phase and weight of the solid phase recorded. The two phases of the rumen and caecum contents were obtained by wrapping a representative sample in surgical gauze and squeezing in a hand press. No attempt was made to separate the phases in the contents of the colon and rectum. All liquid phases were centrifuged at 60,000 g. for 30 mins. before sampling for determination of radioactivity and Mg content.

The walls of the gastro-intestinal tract were washed free from contents, and after the lengths of the sections of small intestine had been measured the mucous membranes were stripped from the walls of the small intestine, caecum, colon and rectum by the method of J.G. Brotherston, N.J.L. Gilmour and J.M. Samuel (1960 Personal Communication). In this method the mucosa was exposed by slitting the wall with bowel scissors and separated from the serosa by scraping with a glass microscope slide. In sheep B the walls of these structures were divided into an anterior and posterior portion. The mucous membranes were stripped from the walls of the rumen and abomasum by hand.

Duplicate samples of kidney, liver, spleen, gastrocnemius muscle, bile and various bones were taken from sheep B. The samples of soft tissue were taken before the alimentary tract was removed from the abdominal cavity to minimize contamination with blood and ingesta.

The radioactivity of the samples was determined with a well-type scintillation counter and a scaling circuit. All determinations were corrected for physical decay of the isotope and were completed on the day of collection. For the solid samples, duplicate tubes were filled to a 5 ml. mark and weighed, and radioactivity and Mg were determined in the contents of each. Duplicate portions of the liquid samples were taken for the determination of radioactivity (5 ml.) and Mg (20 ml.).

To investigate the diurnal variation in the Mg concentration in faeces, samples were collected over 3 h. periods, for a total of 24 h., from four wethers that had been on the same dietary regime as sheep A and B for the previous 15 days.

## RESULTS

### Oral administration of $^{28}Mg$

Table 2 shows the distribution of $^{28}Mg$ and total Mg in the mucosa and contents of the selected sections of the gastro-intestinal tract of sheep A 10 h. after administration. The specific-activity values, as elsewhere, are expressed as percentage dose x $10^2$/mg of Mg in the sample.

The specific activities of the contents of all sections were higher than that of the plasma (1.1), which was expected in view of the poor absorption of Mg from the gut. A fall in the specific activity of a liquid phase relative to that in the previous section of the tract will be due either to secretion of Mg or to the preferential absorption of Mg compounds of a higher specific activity relative to the mean for the liquid phase of the previous section. On the other hand, an increase in the specific activity of a liquid phase can only be due to selective absorption of compounds of a lower specific activity than the mean for the previous section.

The results s u g g e s t that the main sites of secretion into the tract were the abomasum and the first 8 ft. of the small intestine, and the main site of absorption was the middle third section of the small intestine. The fall in specific activity of the liquid phase from the rumen to the abomasum could not arise from a more complete mixing of the active Mg in the liquid phase of the rumen digesta with inactive Mg in the solid phase, because the specific activity of the solid phase of the

Table 2

Distribution of $^{28}Mg$ and total Mg in the contents and walls of the gastro-intestinal tract of sheep A 10 h. after oral administration of $^{28}Mg$

| Site | Length of section (ft) | Mucosa S.A.*‡ | Liquid phase Volume (ml) | Liquid phase Concentration of Mg (mg/100 ml)‡ | Liquid phase S.A.*‡ | Solid phase Dry weight (g) | Solid phase Concentration of Mg (% D.W.)‡ | Solid phase S.A.*‡ | Mean value for liquid and solid phases S.A.*‡ |
|---|---|---|---|---|---|---|---|---|---|
| Rumen | - | 1.36 | - | 4.7 | 5.04 | - | 0.101 | 4.63 | - |
| Abomasum | - | 1.46 | 140 | 8.5 | 3.88 | 11.0 | 0.069 | 3.35 | 3.64 |
| Small intestine: | | | | | | | | | |
| Section 1 | 8 | 1.37 | 45 | 10.0 | 2.96 | 3.0 | 0.063 | 3.16 | 3.02 |
| Section 2 | 12 | 1.68 | 120 | 11.5 | 3.48 | 10.3 | 0.083 | 3.11 | 3.33 |
| Section 3 | 15 | 3.17 | | | | | | | |
| Section 4 | 24 | 1.62 | 125 | 11.5 | 4.04 | 13.1 | 0.125 | 2.79 | 3.37 |
| Section 5 | 11 | 1.41 | 100 | 12.9 | 4.20 | 15.8 | 0.152 | 3.56 | 3.78 |
| Caecum | - | 1.79 | - | 19.5 | 4.15 | - | 0.197 | 3.64 | - |
| Colon: | | | | | | | | | |
| Section 1 | - | 1.90 | - | - | - | - | - | - | 3.78 |
| Section 2 | - | 1.88 | - | - | - | - | - | - | 4.04 |
| Rectum | - | - | - | - | - | - | - | - | 3.83 |

*Specific activity expressed as percentage of dose x $10^2$/mg of total Mg.

‡Mean of two determinations.

abomasum is lower than that of the rumen. Absorption was associated with an increase in the specific activity of the liquid phase, showing that Mg compounds of a relatively low specific activity were preferentially absorbed.

The specific activity of the liquid phase was generally higher than that of the solid phase of the same section. It should be noted that the observed differences are lower than the true values since the solid phase still contained some of the liquid phase. Of more practical importance was the difference between the specific activity of the liquid phase and that of the total contents, since it measures the failure of $^{28}Mg$ administered as chloride to act as a tracer for dietary Mg. It may be seen that the specific activity of the liquid phase was generally higher and that the difference varied with the section (Table 2).

Excluding the third section of the small intestine, which is the main site of absorption, the specific activities of all the mucosa segments of the gastro-intestinal tract were similar and were all higher than the specific activity of the plasma. In theory such an increase may be due to two factors, absorption of $^{28}Mg$ and exchange of $^{28}Mg$ in the intestinal contents with the stable Mg in the mucosa. The latter process is probably the main cause since there was no significant difference in specific activity between the mucosa of the abomasum, which is known to secrete Mg, and those of the other sections.

The concentration of total Mg in the liquid phase showed a progressive increase along the tract from the rumen to the caecum, the last section whose contents were separated.

Intravenous administration of $^{28}Mg$

Walls and contents of the gastro-intestinal tract. - Table 3 shows the distribution of $^{28}Mg$ and stable Mg in the mucosa and contents of the selected segments of the tract of sheep B, 10 h. after administration of $^{28}Mg$. The specific activities of the contents of all sections were lower than that of the plasma, owing to the mixing of endogenous with stable exogenous Mg in the lumen of the gut.

There was an increase in the specific activity of the liquid phase from the rumen to the abomasum and from the abomasum to the first section of the small intestine. It must have been due either to secretion of Mg or to the preferential absorption of Mg compounds of a lower specific activity relative to the mean for the liquid phase of the previous section. Secretion takes place in the abomasum through the gastric juice (Garton, 1951) and in the first section of the small intestine through the bile and pancreatic juice (Field, 1960b), but absorption of Mg of a low specific activity cannot be excluded.

There was a progressive decline in the specific activity of the liquid phase from the mean for sections 2 and 3 to section 6 of the small intestine (as numbered from the pylorus). Since the specific activity of the solid phase did not increase from sections 2 and 3 to 6, there was no evidence of exchange with endogenous Mg of relatively high specific activity, and the fall must therefore have been due to preferential absorption of Mg compounds of high specific activity. The main site of absorption was probably section 4, since the fall from section 3 to 4 was the greatest, i.e. 4.53 to 2.34.

Table 3

Distribution of $^{28}Mg$ and total Mg in the contents and walls of the gastro-intestinal tract of sheep B 10 h. after intravenous administration of $^{28}Mg$

| Site | Length of section (ft) | Mucosa S.A.*‡ | Liquid phase Volume (ml) | Liquid phase Concentration of Mg (mg/100 ml) | Liquid phase S.A.* | Solid phase Dry weight (g) | Solid phase Concentration of Mg (% D.W.) | Solid phase S.A.* | Mean value for liquid and solid phases S.A.* |
|---|---|---|---|---|---|---|---|---|---|
| Rumen | - | 1.96 | - | 2.95 | 0.41 | - | 0.096 | 0.12 | - |
| Abomasum | - | 5.10 | 439 | 3.42 | 1.67 | 22.1 | 0.046 | 0.82 | 1.32 |
| Small intestine: | | | | | | | | | |
| Section 1 | 24 | 8.53<br>6.43 | 104 | 7.30 | 5.07 | 4.2 | 0.066 | 3.50 | 4.65 |
| Section 2 | 8 | 5.00<br>4.45 | 147 | 7.40 | 4.53 | 7.1 | 0.074 | 3.08 | 4.06 |
| Section 3 | 15 | 7.67<br>4.40 | | | | | | | |
| Section 4 | 11 | 3.98<br>- | 93 | 8.22 | 2.34 | 4.17 | 0.121 | 1.56 | 2.03 |
| Section 5 | 10 | 2.82<br>3.07 | 134 | 9.00 | 1.75 | 9.75 | 0.105 | 1.53 | 1.65 |
| Section 6 | 17 | 4.75<br>4.18 | 93 | 8.55 | 1.14 | 3.71 | 0.166 | 1.05 | 1.07 |
| Caecum | - | 3.00<br>3.45 | - | 17.05 | 1.50 | - | 0.150 | 1.30 | - |
| Colon: | | | | | | | | | |
| Section 1 | - | 2.74<br>2.96 | - | - | - | - | - | - | 1.10 |
| Section 2 | - | 3.99<br>5.53 | - | - | - | - | - | - | 1.11 |
| Rectum | - | - | - | - | - | - | - | - | 0.20 |

*Specific activity expressed as percentage of dose x $10^2$ per mg of Mg.

‡The first of the two values applies to the anterior and the second to the posterior part.

A marked variation in the specific activity of the mucosa from organ to organ and from section to section in the same organ was observed. The values for the small intestine, for example, ranged from 2.82 for the first part of section 5 to 8.53 for the first part of section 1. Section 1 and the first part of section 3 had specific activities greater than the plasma (5.70). The specific activity of the mucosa is dependent not only upon absorption and secretion but also upon the rate at which the Mg in the mucosa exchanges with the $^{28}Mg$ in either the plasma or the liquid phase of the ingesta. For this reason no conclusions could be drawn on the cause of the variation in specific activity along the tract.

The specific activity of the liquid phase was always higher than that of the solid phase of the same section. The difference was not a constant from section to section; it was greatest in those sections where secretion was occurring and least in sections 5 and 6 of the small intestine, where reabsorption of intestinal secretions was practically complete.

The pattern of variation in the concentration of total Mg in the liquid phase along the tract was similar to that for sheep A but the values were generally smaller.

Body fluids, soft tissues and bones. - The values obtained for the specific activity and the relative specific activity of the selected soft tissues and body fluids are given in Table 4.

Table 4

Specific activity and relative specific activity of samples of tissues and bile of sheep B

| Sample | S.A.* | R.S.A.‡ | Sample | S.A.* | R.S.A.‡ |
|---|---|---|---|---|---|
| Bile | 7.1 | 1.24 | Femur shaft | 0.048 | 0.0084 |
| Kidney | 6.4 | 1.12 | Femur epiphysis | 0.13 | 0.023 |
| Liver | 5.1 | 0.89 | Rib shaft | 0.079 | 0.014 |
| Spleen | 4.0 | 0.70 | Rib sternal end | 0.13 | 0.023 |
| Muscle | 0.23 | 0.040 | Lumbar vertebra | 0.10 | 0.018 |

*Specific activity expressed as percentage of dose x $10^2$ per mg. of total Mg

‡Relative specific activity = $\frac{\text{S.A. of tissue Mg}}{\text{S.A. of plasma Mg}}$

Relative specific activity is the ratio of the specific activity of the tissue to that of the plasma and is a measure of the proportion of stable Mg that has exchanged during the 10 h. period between administration of $^{28}Mg$ and the death of the sheep. The fact that the values for kidney and bile were greater than 1 may be due to two factors:-

(1) the falling specific activity of the plasma (Care, Macdonald and Nolan, 1959); and

(2) failure of ionic $^{28}Mg$ to exchange with the bound Mg in the plasma, thereby providing a fraction of relatively higher specific activity for exchange with intracellular Mg.

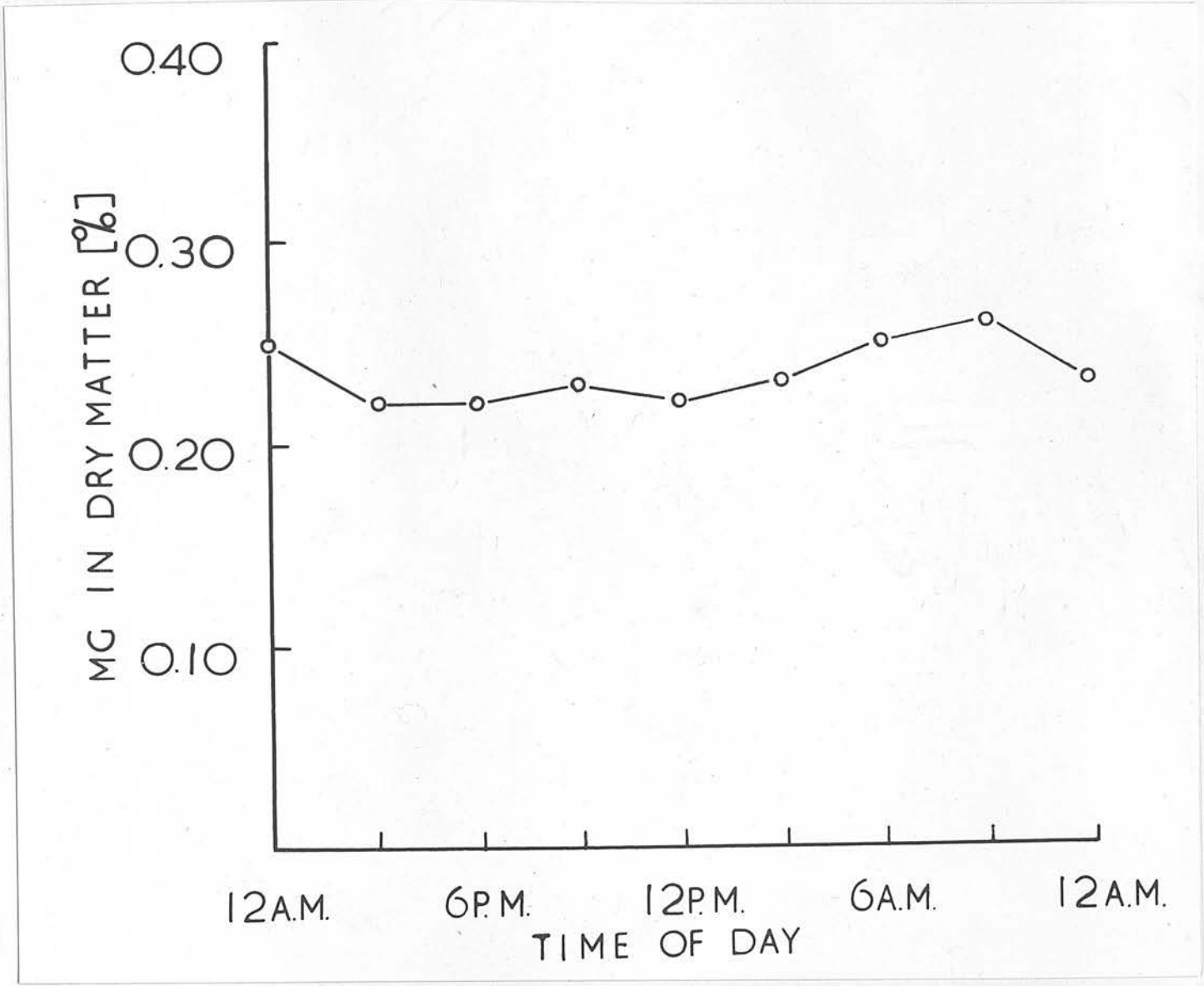
A striking feature was the variation in specific activity in different tissues; the values ranged from 6.4 for kidney to 0.23 for skeleton muscle. Thus the rate at which $^{28}Mg$ exchanges varies with different tissues, and their Mg may exist in different chemical forms.

The specific activities and relative specific activities for the samples of bone are given in Table 4. The over-all proportion of bone Mg in equilibrium with the Mg in body fluids was low, but there was marked variation from bone to bone. It was greater in cancellated than in compact bone. Thus the sternal end of the rib and the epiphysis of the femur showed the greatest exchange and the femur shaft the least. (The uptake of $^{28}Mg$ by the skeleton has been briefly described elsewhere (Field, 1960a).)

Diurnal variation in Mg concentration in faeces

The mean Mg content of the faeces of four wethers over a 24 h. period is shown in Fig. 1. Although the mean values tended to be higher in the morning than in the afternoon, the trend was not significant. Thus there was no evidence for a diurnal variation similar to that reported for calcium and phosphorus in pigs (Moore and Tyler, 1955a) and the results reported above for the distribution of Mg along the tract were therefore independent of the interval between the last feed and the killing of the sheep.

Fig. 1. Diurnal variations in the mean Mg content of the faeces of four sheep.



# SECTION II

## THE EFFECT OF ABRUPT CHANGES IN THE NATURE OF THE DIET ON THE URINARY MAGNESIUM EXCRETION OF SHEEP

## EXPERIMENTAL

### Animals

Adult wethers of various ages, weights and breeds were used as experimental animals. Sheep A to D were 4-year-old North Country Cheviots, E was a 4-year-old Greyface, F a 4-year-old Suffolk, and G and H were 3-year-old Blackfaces. Their weights ranged from 55 kg. for the Blackfaces to 70 kg. for the Suffolk. Before the beginning of the experiments all sheep were accustomed to being harnessed and crated.

### Rations

Since a high incidence of hypomagnesaemic tetany in dairy cows has been associated with the grazing of fertilized leys in the spring, this type of herbage was used in these experiments. One sample ($S_4$) was collected from a pasture on which the disease had occurred in lactating beef cows and ewes 2 days previously, and the other three were from ungrazed pastures. Samples were collected during May and June 1956 and April 1957 and they were stored in a local cold store at $-15^{\circ}$ or $-20^{\circ}$ until required for use. Details of the chemical and botanical composition, the fertilizer treatment and the age of the sward are given in Table 5.

The hay and grass nuts used were bought from a local merchant and their origin is unknown. The protein content (13%) and digestibility coefficient (about 45%) of the two samples of grass nuts suggest, however,

Table 5

Details of diet of the sheep

| Ration | Month and year of collection | Type of sward | Fertiliser treatment* | Dominant species† | Dry matter (%) | Mg in dry matter (%) |
|---|---|---|---|---|---|---|
| S1 | June 1956 | 2nd-year ley | C | P.R.G., C., W.W.C. | 20.4 | 0.162 |
| S2 | May 1957 | 1st-year ley | C | P.R.G. | 14.2 | 0.274 |
| S3 | May 1957 | 2nd-year ley | S.A., K. | P.R.G., C., W.W.C. | 17.7 | 0.171 |
| S4 | June 1957 | 1st-year ley | P | I.R.G., P.R.G., C., B.R.C. | 16.0 | 0.108 |

*P., potato manure (10% N, 10% $P_2O_5$, 15% $K_2O$); C., compound (8% N, 9.5% $P_2O_5$, 13% $K_2O$); S.A., sulphate of ammonia; K., potassium superphosphate (15.5% $P_2O_5$, 10% $K_2O$).

†I.R.G., Italian rye grass; P.R.G., perennial rye grass; C., cocksfoot; B.R.C., broad red clover; W.W.C., wild white clover.

that they were not made from spring herbage. Sufficient chopped hay or grass nuts for an experiment was mixed and sampled for chemical analysis, and the requisite amounts, either 1 or 1½ lb. were weighed out into Polythene bags.

Design of experiments

Each experiment comprised two or three successive periods of varying duration (9-18 days) during which the intake and urinary excretion of Mg were measured. No attempt was made to measure the faecal excretion, since after a change of diet there is a delay of 5 or 6 days before the faeces are representative of the new diet. Table 6 gives details of each experiment in terms of the sheep used, the nature and Mg content of the diet in each feeding period and the length of the period. In Expt. 1 the change in diet from grass nuts ($GN_1$) to spring herbage ($S_1$) involved a reduction in Mg intake of 0.5 g/day, whereas the dietary change from hay ($H_1$) to spring herbage ($S_2$) in Expt. 2 involved an increase in Mg intake of 0.66 g/day. Expt. 3 was a repetition of Expt. 2 with different sheep and diets and in addition was designed to investigate the reverse change in diet. Expt. 4 represented an unsuccessful attempt to produce hypomagnesaemia in two wethers by an abrupt change from hay ($H_1$) to a spring herbage ($S_4$) collected from a pasture on which hypomagnesaemic tetany had occurred 2 days previously in lactating beef cows and ewes, and which had a very low magnesium content supplying only 0.94 g/day. After $S_4$ had been given for 18 days, the diet was changed back to the original hay. The differences between the figures given in Table 6 for

## Table 6

## Design of experiments

| | | Feeding period | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | | | 2 | | | 3 | | |
| Expt. No. | Sheep used | Length of period (days) | Diet* | Mg intake (g/day) | Length of period (days) | Diet* | Mg intake (g/day) | Length of period (days) | Diet* | Mg intake (g/day) |
| 1 | A, B | 9 | $GN_1$ | 2.28 | 9 | $S_1$ | 1.78 | - | - | - |
| 2 | A, C | 9 | $H_1$ | 1.46 | 9 | $S_2$ | 2.12 | - | - | - |
| 3 | E, F, G, H | 9 | $GN_2$ | 1.10 | 9 | $S_3$ | 1.65 | 9 | $GN_2$ | 1.10 |
| 4 | A, D | 9 | $H_1$ | 1.30 | 18 | $S_4$ | 0.94 | 9 | $H_1$ | 1.30 |

*GN, grass nuts; H, hay; S, spring herbage.

the Mg intake when the same hay was given are due to variation between bales and to sampling and analytical errors. A ration similar in nature to the one used in the first period was given to each sheep for at least a month before the beginning of an experiment to allow time for the animal's metabolism to become adapted to the diet.

The contents of one food bag were given at 10 a.m. and of the other at 4 p.m. and were eaten completely. Distilled water was always available in plastic containers during the experiments. On the day before the ration was changed to spring herbage, each sheep was passively immunized against pulpy-kidney disease.

Daily quantitative collections of urine were made for the last 6 days of the first period and were continued to the end of each experiment. The urine was collected into a Polythene bottle containing 100 ml. of 50% (v/v) glacial acetic acid (AR). After the volume of urine and acid had been measured, about 250 ml. were filtered through nylon cloth and one-tenth of the total volume was taken for chemical analysis. At the end of the collection period, the daily samples were taken for Ca and Mg estimation.

At intervals throughout the experiments blood samples were taken from the jugular vein for the estimation of serum Mg levels.

## RESULTS

### Clinical condition of the sheep

No clinical abnormalities were observed in the sheep at any time during the experiments.

### Serum Mg levels

The values are given in Table 6a.

Throughout the experiments the concentration of Mg in the serum remained within the generally accepted normal range (2-4 mg/100 ml), although herbage from a field ($S_4$) on which tetany occurred was given to sheep A and D for 18 days. Further, none of the dietary changes studied produced any marked change in the serum Mg level within this normal range. For these reasons the values obtained are not recorded here.

### Urinary Mg excretion (UMg)

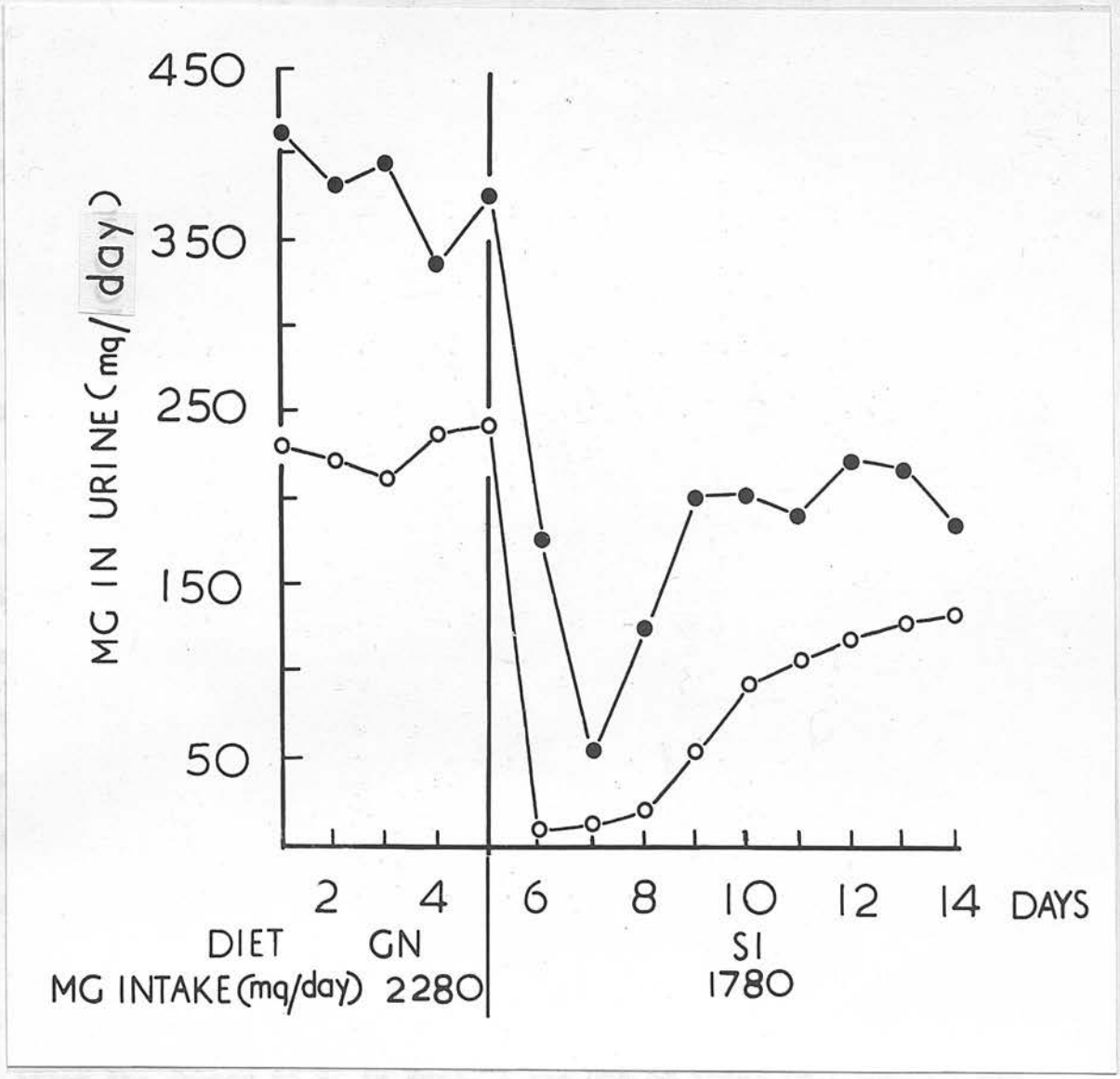
In the first experiment, the change in diet from grass nuts ($GN_1$) to spring herbage ($S_1$) was followed immediately by a fall in UMg with minimal values occurring on the 1st day in sheep A and on the 2nd day in sheep B. Thereafter the UMg of both sheep rose; that of sheep A was still rising, though less rapidly, at the end of the observation period of 9 days, but that of sheep B reached a constant value on the 4th day after the dietary change (Fig. 2). These rises which occurred when the Mg intake (DMg) remained constant suggested that the reduction

Table 6a

Concentration of Mg in the serum of the sheep (mg/100 ml)

| Expt. no. | No. of sheep | Day of experiment | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 18 | 22 | 26 | 30 |
| 1 | A | 3.13 | 3.07 | 3.13 | 3.33 | 3.07 | 3.33 | 3.67 | | | | |
| | B | 2.87 | 2.60 | 3.00 | 2.87 | 2.93 | 3.07 | 3.53 | | | | |
| 2 | A | 2.93 | 3.07 | 3.07 | 3.33 | 3.07 | 3.13 | 3.47 | | | | |
| | C | 2.50 | 2.73 | 2.87 | 3.00 | 2.47 | 2.87 | 2.80 | | | | |
| 3 | E | 2.80 | 2.60 | 3.00 | 2.93 | 3.07 | 3.07 | 2.87 | 2.95 | 3.00 | | |
| | F | 2.55 | 2.73 | 3.00 | 3.07 | 2.60 | 3.07 | 3.13 | 2.95 | 2.80 | | |
| | G | 2.20 | 2.05 | 2.15 | 2.30 | 2.25 | 2.15 | 2.15 | 2.05 | 2.25 | | |
| | H | 2.45 | 2.35 | 2.45 | 2.55 | 2.55 | 2.35 | 2.35 | 2.40 | 2.40 | | |
| 4 | A | 3.07 | 3.13 | 3.13 | 3.33 | 3.25 | 2.87 | 3.07 | 3.00 | 3.13 | 3.13 | 3.13 |
| | D | 2.60 | 2.65 | 2.55 | 2.70 | 2.55 | 2.65 | 2.40 | 2.55 | 2.72 | 2.65 | 2.55 |

Fig. 2. Effect of a dietary change from grass nuts ($GN_1$) to spring herbage ($S_1$) on the UMg of two sheep. O, sheep A; ●, sheep B.



in DMg associated with the dietary change was not the sole cause of the initial fall in UMg.

The dietary change from hay ($H_1$) to spring herbage ($S_2$) in Expt. 2 was followed immediately by a fall in the UMg of both sheep; minimal values occurred on the 2nd day after the change for sheep A and on the 1st day for sheep C (Fig. 3). Thereafter the UMg of sheep A rose until the 5th day when it reached a constant value, which was similar to that when diet $H_1$ was given. Thus, by the end of the observational period, the UMg had not reflected the increased DMg. With sheep C, the initial fall was followed by a marked increase to values much higher than those obtained with diet $H_1$, but thereafter the UMg again fell sharply.

After the dietary change from grass nuts ($GN_2$) to herbage ($S_3$) in Expt. 3, three of the four sheep used, F, G and H, showed a similar picture to that obtained with sheep A in Expt. 2, namely, an initial fall in UMg followed by an increase to values similar to those obtained when the previous diet containing less Mg was given (Fig. 4). The other sheep (E) showed no initial fall and the UMg increased to higher values than those obtained during the first period. The reverse change of diet from $S_3$ to $GN_2$ produced an initial increase in UMg in all sheep despite the fall in DMg. Thereafter UMg fell to values similar to those obtained previously when diet $GN_2$ was given.

After the change to $S_4$ in Expt. 4 the UMg at first fell sharply and then remained relatively constant at a low level until the diet was changed back to $H_1$, when a marked increase followed by a rapid fall to very low values (6 and 13 mg/day for sheep A and D respectively) occurred (Fig. 5).

Fig. 3. Effect of a dietary change from hay ($H_1$) to spring herbage ($S_2$) on the UMg of two sheep. O, sheep A; ●, sheep C.

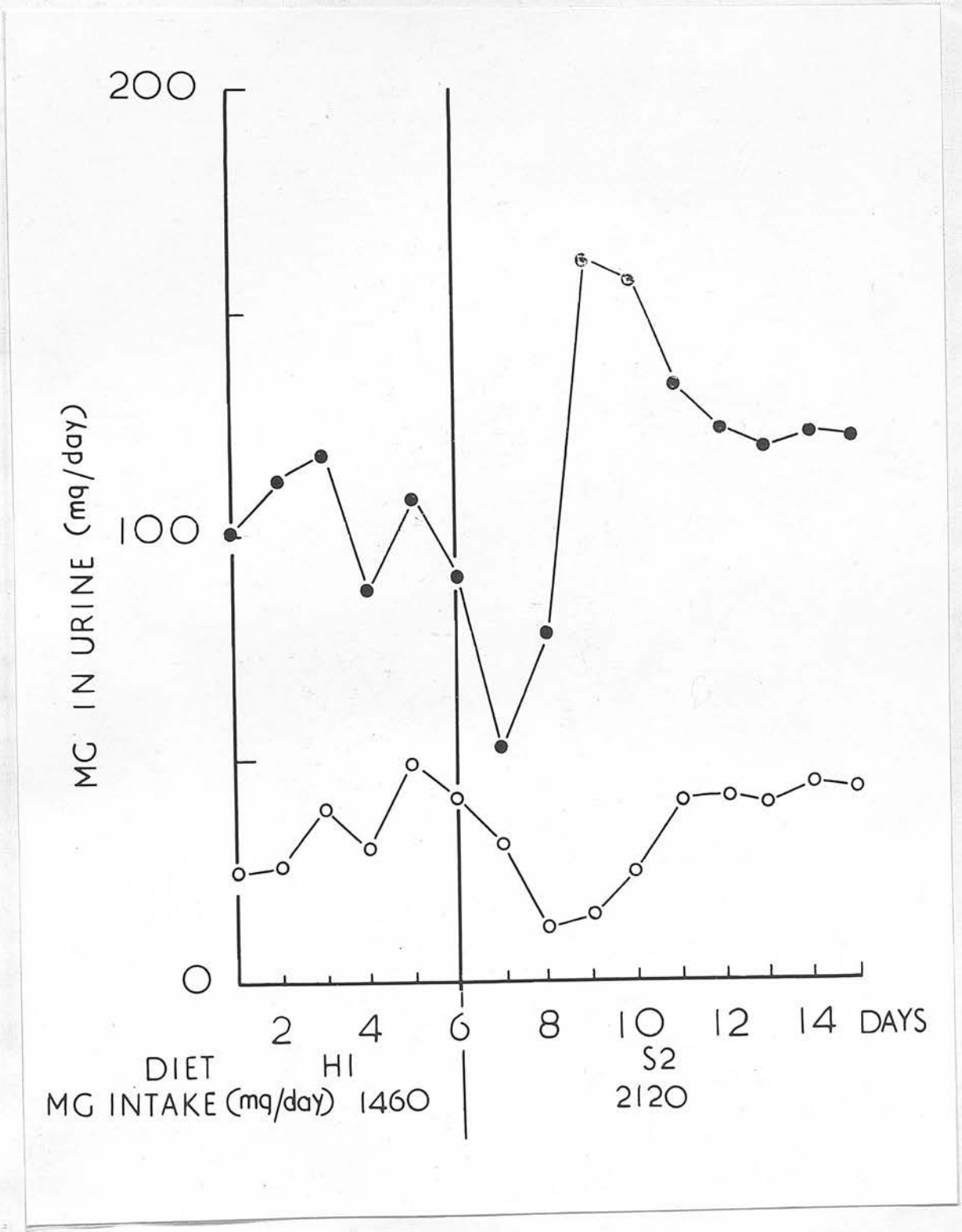


Fig. 4. Effect of dietary changes from grass nuts ($GN_2$) to spring herbage ($S_3$) and back to grass nuts ($GN_2$) on the UMg of four sheep. O--O, sheep E; O — O, sheep F; ●, sheep G; +, sheep H.

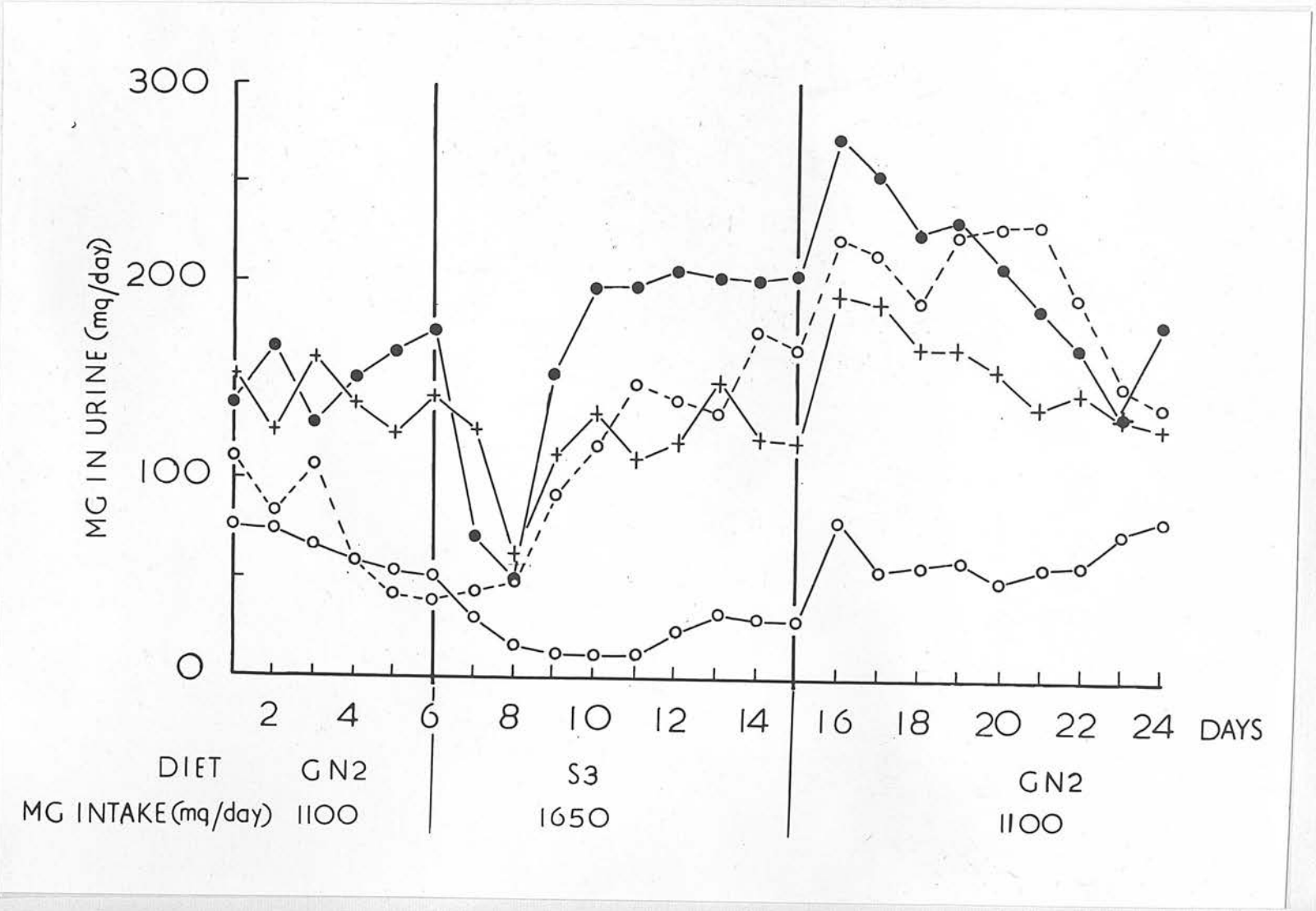
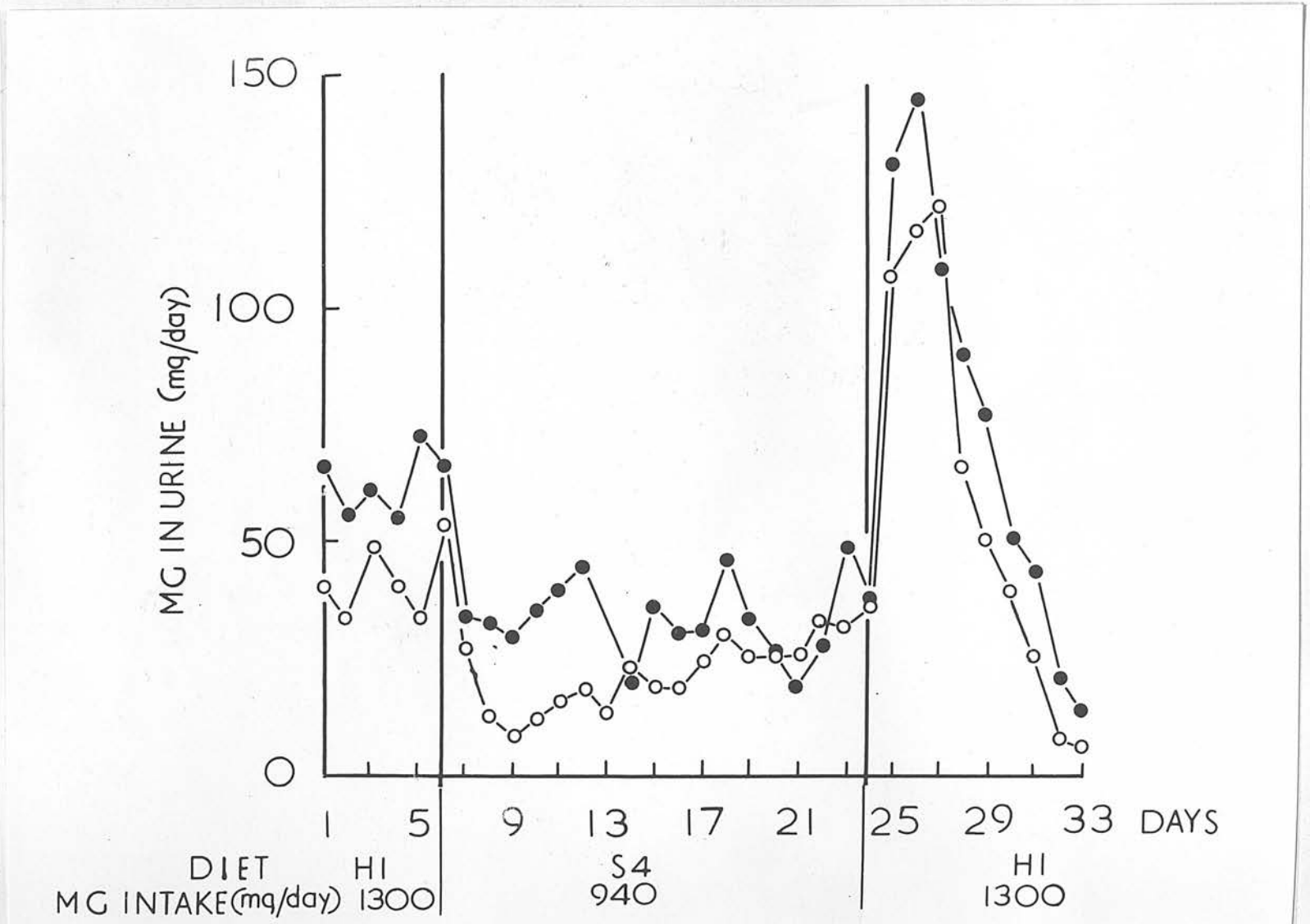


Fig. 5. Effect of dietary changes from hay ($H_1$) to spring herbage ($S_4$) and back to hay ($H_1$) on the UMg excretion of two sheep. O, sheep A; ●, sheep D.

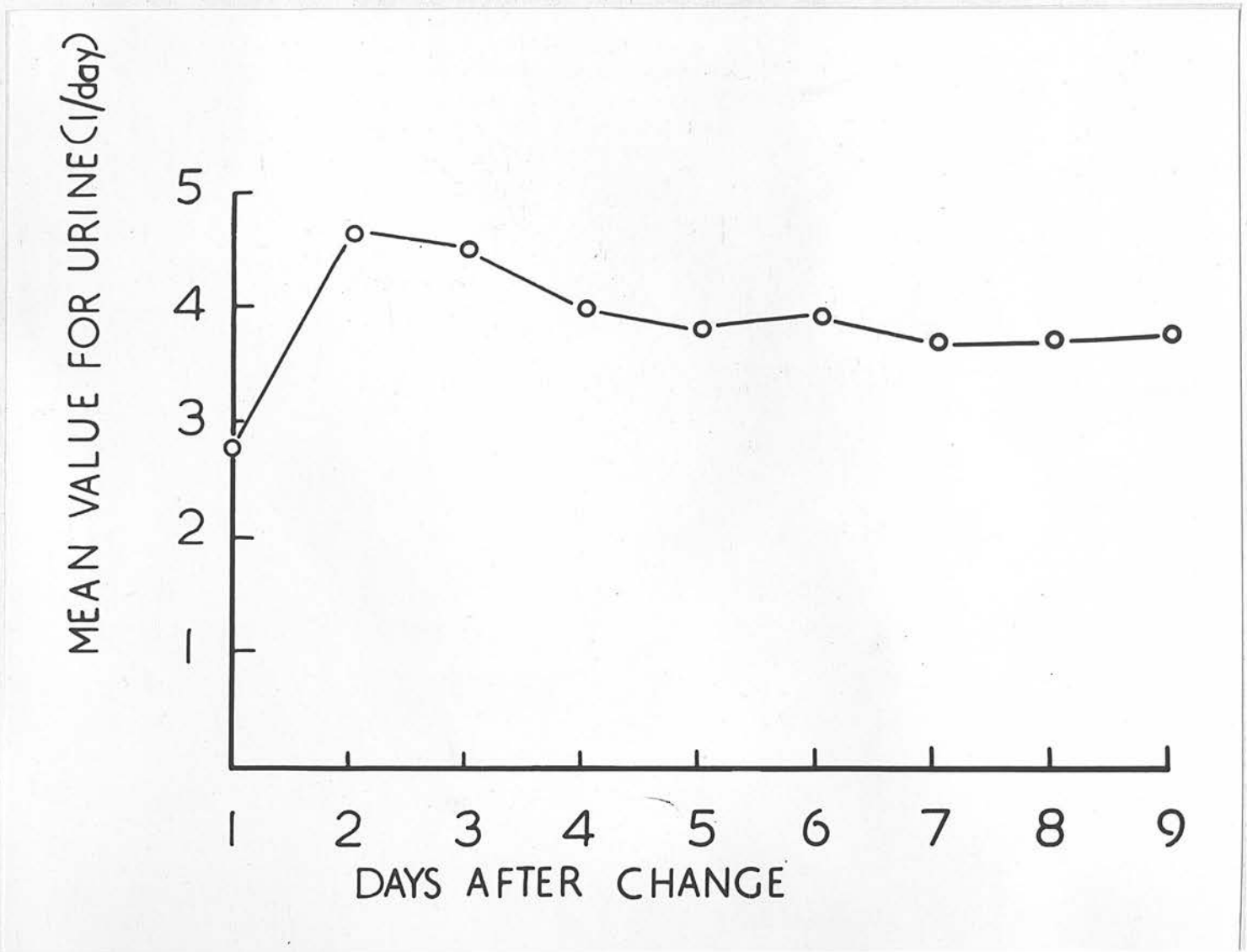


The maximum values during this third period, 120 and 144 mg/day for sheep A and D respectively, were higher than those obtained in the first period on this diet and occurred for sheep A on the 3rd day and for sheep D on the 2nd day after the diet had been changed. This result was similar to that obtained in Expt. 3, but the peak in the excretion curve was more exaggerated, possibly owing to the longer feeding of spring herbage and to the increase in DMg associated with this dietary change.

Volume of urine

The time course of changes in the mean volume of urine excreted by all the sheep whose diet was changed from hay or grass nuts to spring herbage is plotted in Fig. 6. The pattern was similar for all sheep, except that the largest volume was voided on the 2nd day after the diet had been changed by some sheep and on the 3rd day by the remainder. At these times the values for urinary Mg excretion were at their lowest. There were no gross differences in urine volume from sheep to sheep on the same experiment.

Fig. 6. The time course of changes in the mean value of urine excreted by all sheep whose diet was changed from hay or grass nuts to spring herbage.



# SECTION III

## BALANCE TRIALS WITH SHEEP GIVEN DIFFERENT AMOUNTS OF GRASS NUTS

## EXPERIMENTAL

The experiments were carried out with four apparently healthy North Country Cheviot wethers (referred to as sheep A, B, C and D), aged about 4 years at the beginning of the experiments and weighing 59, 75, 66 and 68 kg. respectively. Sheep B had been used in a previous experiment (Field et al., 1958). The sheep were harnessed for the separate quantitative collection of urine and faeces and housed in individual cages.

Two experiments were conducted at an interval of about 1 year. Each comprised a series of balance trials in which the sheep were given 900, 1200 or 1500 g. artificially dried grass in the form of nuts each day over successive periods of 15 days. The sequence in which these amounts were given was the same for each sheep. Each trial was made up of a 9-day preliminary and a 6-day collection period. A replicate balance trial at the 1200 g. level of intake was carried out in the first experiment to obtain an estimate of reproducibility. The sheep were fed twice daily with equal portions at 10 a.m. and 4 p.m. and distilled water was always available.

The grass nuts were purchased in one batch from the local merchant, thoroughly mixed and stored in galvanized steel bins until required. Their composition is given in Table 7.

Table 7

Percentage composition of nuts made from artificially dried grass

| | |
|---|---|
| Moisture | 11.3 |
| Crude protein | 8.6 |
| Ether extract | 3.1 |
| Crude fibre | 29.1 |
| Nitrogen-free extractives | 37.7 |
| Ash | 10.2 |
| Magnesium | 0.128 |
| Calcium | 0.644 |

Faeces and urine were collected over 24 h. periods, the faeces into Polythene bags and the urine into a Polythene bottle containing 100 ml. of 50% (v/v) glacial acetic acid (AR). After the volume of urine and acid had been measured, about 250 ml. were filtered through nylon cloth and one-tenth of the total volume was taken for chemical analysis. At the end of the collection period, the daily samples were pooled, and duplicate samples were taken for Ca and Mg estimation. The faeces were weighed daily and transferred to a Polythene bowl. After thorough mixing, one-fifth was weighed out into a Polythene bag which was closed with a rubber band and stored at 4°. Samples taken over the whole period were pooled in this bag. The composite sample was then divided into three approximately equal portions which were dried separately in an electric oven at 100°-105° and ground in a Christy Norris mill with a 1 mm. sieve.

At 11 a.m. on the last day of each trial, samples of serum were taken for Ca and Mg estimation.

Table 8

Intake and excretion of magnesium by the sheep
(mean daily values for each collection period)

| | | Sheep A | | Sheep B | | Sheep C | | Sheep D | |
|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Intake (g) | U*(mg) | F*(g) | U (mg) | F (g) | U (mg) | F (g) | U (mg) | F (g) |
| | 1.02 | 97 | 0.94 | 24 | 1.04 | 52 | 1.04 | 106 | 0.98 |
| 1 | 1.36 | 112 | 1.28 | 36 | 1.37 | 74 | 1.49 | 152 | 1.19 |
| | 1.70 | 147 | 1.49 | 53 | 1.73 | 126 | 1.65 | 190 | 1.44 |
| | 1.02 | 77 | 0.87 | 32 | 0.86 | 32 | 0.98 | 96 | 0.91 |
| 2 | 1.36 | 103 | 1.11 | 36 | 1.23 | 62 | 1.20 | 135 | 1.16 |
| | 1.70 | 127 | 1.50 | 47 | 1.61 | 72 | 1.60 | 166 | 1.50 |

Standard deviations (6 DF) of urinary and faecal excretion are 7 mg and 0.056 g/day respectively.

*U and F stand respectively for urinary and faecal excretion.

## RESULTS

### Urinary magnesium excretion

The mean daily values for urinary Mg excretion (UMg) are given in Table 8, together with the daily intakes (DMg) to which they are clearly related. The regression coefficients of UMg on DMg differed significantly between sheep ($P<0.01$), but not between experiments, whereas the constant terms in the regression equations differed both between sheep ($P<0.005$) and between experiments ($P<0.005$). Values for the regression coefficients and constant terms are given in Table 9.

The second order interactions (sheep x experiment x level of feeding) have been used as the estimates of error for carrying out significance tests for each of the measurements analysed.

Table 9

Values for the constants b and c in the regression equations UMg = b DMg + c (mg)

| | Sheep A | Sheep B | Sheep C | Sheep D |
|---|---|---|---|---|
| b (both experiments) | 0.074 | 0.032 | 0.084 | 0.113 |
| c (Expt. 1) | 16.8 | 0.4 | -37.9 | -6.5 |
| c (Expt. 2) | 4.2 | -12.2 | -50.5 | -19.1 |

The standard error of b is $\pm$ 0.010 and that of c is $\pm$ 14

Faecal magnesium excretion

The mean daily values for faecal Mg excretion (FMg) are also given in Table 8 and these too are clearly related to daily intake (DMg). There were some indications in the first experiment of a non-linear relationship, but the effect was small and of no great practical significance. Ignoring this, the regression coefficient of FMg on DMg was 0.882 and did not differ significantly between sheep or between experiments. There were significant differences in the constant terms of the regression equations, and these constants are given in Table 10.

Table 10

Values for the constant c in the regression equation FMg = 0.882 DMg + c (mg)

| | Sheep A | Sheep B | Sheep C | Sheep D |
|---|---|---|---|---|
| Expt. 1 | 60 | 160 | 180 | 90 |
| Expt. 2 | -60 | 40 | 60 | -30 |

The standard error of the constant term is $\pm$ 80

The error due to sampling and chemical analysis, estimated as the variance between the triplicate samples, formed only a small part of the total error.

## Table 11

Mean daily magnesium intake (g) of the sheep, and net magnesium absorption (g) and balance (g) for each collection period

| | | Sheep A | | Sheep B | | Sheep C | | Sheep D | |
|---|---|---|---|---|---|---|---|---|---|
| Expt. No. | Intake | Net absorption | Balance | Net absorption | Balance | Net absorption | Balance | Net absorption | Balance |
| | 1.02 | + 0.08 | - 0.02 | - 0.02 | - 0.04 | - 0.02 | - 0.07 | + 0.04 | - 0.07 |
| 1 | 1.36 | + 0.08 | - 0.03 | - 0.01 | - 0.05 | - 0.13 | - 0.20 | + 0.17 | + 0.02 |
| | 1.70 | + 0.21 | + 0.06 | - 0.03 | - 0.08 | + 0.05 | - 0.07 | + 0.26 | + 0.07 |
| | 1.02 | + 0.15 | + 0.07 | + 0.16 | + 0.12 | + 0.04 | 0.00 | + 0.11 | + 0.01 |
| 2 | 1.36 | + 0.25 | + 0.15 | + 0.13 | + 0.09 | + 0.16 | + 0.09 | + 0.20 | + 0.07 |
| | 1.70 | + 0.20 | + 0.07 | + 0.09 | + 0.04 | + 0.10 | + 0.03 | + 0.20 | + 0.03 |

Standard deviations (6 DF) of net absorption and balance are 0.083 and 0.044 g/day respectively.

Magnesium balance and net absorption

The estimates of the mean daily balance and net absorption are given in Table 11, which shows that the net absorption was generally lower in the first than in the second experiment, and that difference in net absorption occurred between sheep when the Mg intake was the same. These differences were significant ($P<0.01$) but there were no significant differences between the mean net absorptions at different levels of intake. Furthermore, net absorption and urinary excretion were not correlated.

The net absorption is the arithmetic difference between the estimates of intake and faecal excretion of Mg. It is, therefore, subject to the same errors as the latter, but since the absorbed fraction is much less than the faecal fraction, the coefficient of variation for estimates of net absorption becomes much greater than that for faecal estimates. (The coefficient of variation was 53.7%.)

The mean balances for the first and second experiments were significantly different ($P<0.01$) at -0.040 and +0.064 g/day respectively.

Serum magnesium

The serum Mg values are given in Table 12.

Table 12

Concentration of Mg in the serum of the sheep (mg/100 ml)

| Expt. no. | Intake (g/day) | Sheep A | Sheep B | Sheep C | Sheep D |
|---|---|---|---|---|---|
| | 1.02 | 2.47 | 2.13 | 2.73 | 2.40 |
| 1 | 1.36 | 2.67 | 2.55 | 3.13 | 3.13 |
| | 1.70 | 2.73 | 2.73 | 2.55 | 2.40 |
| | 1.02 | 2.35 | 2.13 | 2.35 | 3.07 |
| 2 | 1.36 | 2.40 | 2.50 | 2.67 | 3.07 |
| | 1.70 | 2.27 | 2.05 | 2.08 | 2.25 |

Standard deviation (6 DF) is 0.155 mg/100 ml

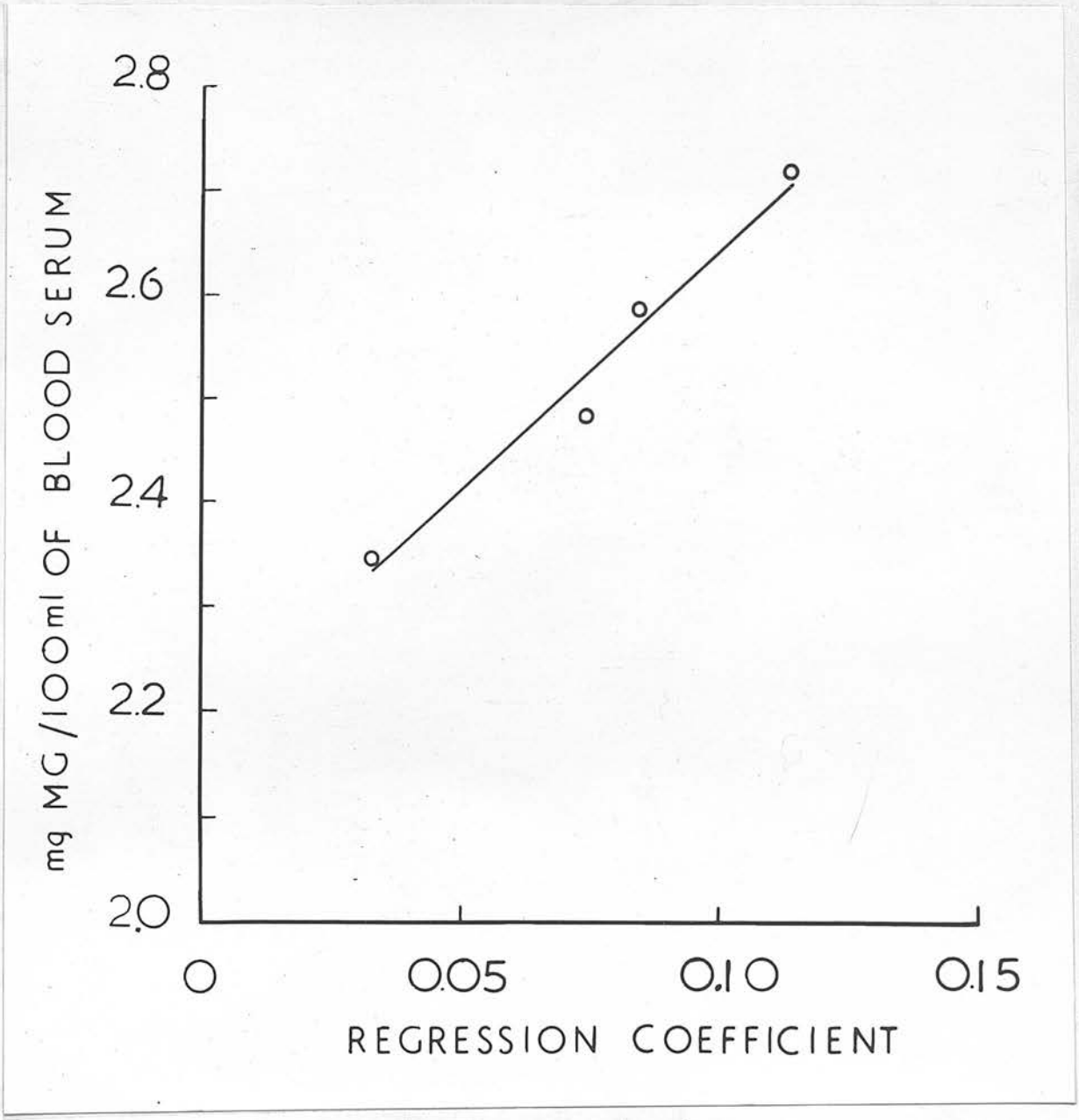
Significant differences were found between sheep; at the intermediate level of Mg intake the values were significantly higher than at the extreme levels and the mean for the first experiment was significantly higher than that for the second, the values being 2.63 and 2.53 mg/100 ml respectively.

The sheep means were significantly correlated ($r = 0.984$, $P < 0.05$) with the coefficients for the regression of UMg on DMg. The relationship can be expressed as follows:-

$$S\,Mg + 4.644\,b + 2.183$$

where S Mg represents the mean serum Mg and b the regression coefficient. The equation and the values obtained experimentally are plotted in Fig. 7.

Fig. 7. Relationship between individual mean serum magnesium levels and the coefficient for the regression of urinary excretion on intake.



Urinary calcium excretion

No systematic differences were found between the respective values for the mean daily urinary excretion of Ca in the two experiments and they may be considered as replicates. The results are given in Table 13.

Table 13

Mean daily urinary calcium excretion (mg) of the sheep

| Expt. no. | Intake (g/day) | Sheep A | Sheep B | Sheep C | Sheep D |
|---|---|---|---|---|---|
| 1 | 5.14 | 35 | 24 | 35 | 14 |
| | 6.86 | 87 | 55 | 85 | 27 |
| | 8.58 | 159 | 97 | 171 | 30 |
| 2 | 5.14 | 29 | 52 | 53 | 18 |
| | 6.86 | 50 | 41 | 70 | 22 |
| | 8.58 | 126 | 95 | 145 | 48 |

The observed increase in urinary Ca with increasing Ca intake was curvilinear ($P<0.005$) for sheep A, B and C. There was no correlation between the urinary excretion of Ca and Mg.

Faecal calcium excretion

The estimated mean daily values for faecal Ca excretion are given in Table 14, which shows that the values for the first experiment were generally lower than the corresponding ones in the second; differences existed between the sheep when the intake was the same, and, as expected, the faecal excretion varied with intake.

## Table 14

### Mean daily calcium intake (g), faecal calcium excretion (g), calcium net absorption (g) and balance (g) of the sheep

| Expt. No. | Intake | Sheep A | | | Sheep B | | | Sheep C | | | Sheep D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F* | A* | B* | F | A | B | F | A | B | F | A | B |
| 1 | 5.14 | 5.48 | -0.34 | -0.37 | 5.57 | -0.43 | -0.45 | 6.22 | -1.08 | -1.11 | 6.49 | -1.35 | -1.36 |
| | 6.86 | 7.05 | -0.19 | -0.28 | 7.37 | -0.51 | -0.56 | 7.64 | -0.78 | -0.86 | 7.42 | -0.56 | -0.59 |
| | 8.58 | 8.76 | -0.18 | -0.34 | 9.04 | -0.46 | -0.56 | 9.44 | -0.86 | -1.03 | 8.54 | +0.04 | +0.01 |
| 2 | 5.14 | 4.46 | +0.68 | +0.65 | 4.20 | +0.94 | +0.89 | 5.87 | -0.73 | -0.78 | 5.90 | -0.76 | -0.78 |
| | 6.86 | 6.23 | +0.63 | +0.58 | 6.95 | -0.09 | -0.13 | 6.52 | +0.34 | +0.27 | 7.14 | -0.28 | -0.30 |
| | 8.58 | 8.18 | +0.40 | +0.27 | 8.87 | -0.29 | -0.39 | 8.37 | +0.21 | +0.07 | 8.75 | -0.17 | -0.22 |

Standard deviations (6 DF) of faecal excretion, net absorption and balance are 0.319, 0.318 and 0.276 g/day respectively.

*F, A and B stand respectively for faecal excretion, net absorption and balance.

The differences were significant ($P<0.01$) and independent. The mean values for the first and second experiments were respectively 7.42 and 6.79 g/day, a mean difference of 0.63 g. or 9.2% of the mean Ca intake.

The relationship between faecal excretion and intake was investigated with the pooled values from the two experiments and found to be rectilinear ($P<0.005$) with no evidence of curvilinearity. The regression coefficient for all sheep was 0.936.

Since Visek, Monroe, Swanson and Comar (1953) have found with cattle that the endogenous faecal Ca excretion is a constant, it can be shown that the proportion of Ca in grass nuts unavailable to the animal was a constant over the range of calcium intakes investigated and equal to the regression coefficient of faecal Ca on dietary Ca. This value, 0.936, was the same for all the sheep and represents an availability of 6.4 per cent.

Calcium balance and net absorption

The results obtained for the mean daily Ca balance and net absorption are given in Table 14. A marked feature of the values for the net absorption was the large sampling and analytical error associated with them; the standard error was $\pm$ 0.216 g/day for each value calculated from the variation between the triplicate samples of faeces and the 95% confidence limits $\pm$ 0.933 g/day. With two exceptions none of the net absorption figures were significantly different from zero, but they showed non-random variation between sheep and between experiments. These differences were significant (sheep $P<0.05$, experiments $P<0.01$).

The values for the net absorption of the sheep were correlated with neither the urinary Ca excretion nor the net absorption of Mg.

On statistical analysis the results for the balance gave similar results to those for net absorption, but the difference between experiments was slightly greater at 0.74 g/day or 10.8% of the average Ca intake.

Serum calcium

The values obtained for the serum Ca concentration are given in Table 15. The only noteworthy feature was that in the first experiment the mean serum Ca value of the sheep decreased with increasing intake, whereas the opposite occurred in the second experiment.

Table 15

Concentration of Ca in the serum of the sheep (mg/100 ml)

| Expt. no. | Intake (g/day) | Sheep A | Sheep B | Sheep C | Sheep D |
|---|---|---|---|---|---|
| | 5.14 | 8.94 | 10.18 | 10.38 | 9.35 |
| 1 | 6.86 | 9.35 | 9.97 | 9.77 | 9.14 |
| | 8.58 | 8.52 | 8.31 | 9.56 | 8.31 |
| | 5.14 | 8.83 | 9.35 | 9.77 | 10.80 |
| 2 | 6.86 | 9.77 | 10.60 | 10.20 | 10.60 |
| | 8.58 | 11.00 | 11.30 | 11.20 | 11.20 |

# DISCUSSION

## DISCUSSION

### Transfer of Magnesium across the wall of the gastro-intestinal tract

Since only a portion of the dietary intake of magnesium exchanges with the total body pool of magnesium, the kinetics of absorption and secretion of magnesium in the intestinal tract are best explained in terms of a two-compartment system, one exchanging with the body pool, the other representing unabsorbed dietary magnesium. The physiologists are interested mainly in the changes in the exchangeable compartment along the tract, whereas the nutritionist is interested in the net changes in the two compartments.

A change in the amount of magnesium in a particular section of the gastro-intestinal tract, other than those associated with the passage of ingesta along the tract, is not the consequence of a one-way passage of magnesium across the gut wall. Rather, it is the consequence of two simultaneous fluxes across the intestinal mucosa, one from the blood to the lumen, the other from lumen to blood. When the flux out of the lumen is greater than that into the lumen, absorption is said to take place. Similarly, secretion results from a greater flux into than out of the lumen.

The simultaneous two-way passage of magnesium across the gut wall was first demonstrated by Stewart and Moodie (1956) but they made no mention of it. They reported absorption by the abomasum, an organ which through the gastric juice is known to be a significant site of secretion of magnesium, when they raised the magnesium concentration in the lumen to

abnormally high concentrations by oral dosing or local injection of inorganic magnesium salts.

Further evidence for a simultaneous two-way passage of magnesium across the gut wall of all sections of the gastro-intestinal tract was obtained in Section I where it was shown that, when $^{28}Mg$ was orally administered, the specific activities of the gut wall exceeded that of the plasma even in those sections where secretion is evidenced by a progressive decline in the specific activity of the liquid phase of the ingesta (Table 2).

Absorption

Site of absorption. - When the magnesium content in the gastro-intestinal tract of sheep was raised to abnormal levels with large doses of magnesium salts, Stewart and Moodie (1956) found that, although absorption occurred from the rumen to the caecum, the main site was the small intestine. It is questionable, however, how far conclusions from such high concentrations may apply to those of the normal range.

Keeping the magnesium ion at normal concentration in the gut, I was able to confirm in Section I the findings of Stewart and Moodie (1956) in one respect by comparing the specific activities of the liquid phase of the ingesta along the tract after oral or intravenous administration of single doses of $^{28}Mg$. My observations showed clearly that the main site of magnesium absorption is the middle third of the small intestine and, although the differences between the specific activities of the mucosa of the tract and the plasma after oral administration are consistent

with a limited absorption along the whole tract from the rumen to the colon, no definite conclusions could be drawn regarding other sites because of the complicating factor of isotopic exchange.

Determination of availability of dietary magnesium and endogenous faecal magnesium excretion. -

Studies of the absorption of magnesium are complicated by the fact that the magnesium in the gastro-intestinal tract contains unabsorbed magnesium derived not only from the food but also from the intestinal secretions and that it is very difficult to determine the relative proportions of these two fractions.

Field (1959) and MacDonald et al. (1959) attempted to do this with $^{28}Mg$ using methods originally developed for $^{45}Ca$, the comparative-balance (Hansard et al., 1954), and the isotope-dilution (Visek et al., 1953) respectively, but because of the good agreement between the values obtained with the two techniques there has been a tendency to overlook the assumptions inherent in them.

In the comparative-balance technique $^{28}Mg$ in the ionic form is used as a tracer for dietary magnesium, despite the fact that not all the magnesium in the food is in the ionic form. Theoretically, $^{28}Mg$ should be incorporated in, and allowed to equilibrate with, the magnesium of the diet before feeding. The short half life of $^{28}Mg$ prevents this, so that the only possibility is to give $^{28}Mg$ orally in the hope that before absorption the $^{28}Mg$ and inactive dietary magnesium will mix and exchange homogenously in the rumen. If such distribution does not occur the

figures for the availability of dietary magnesium will be more applicable to the availability of ionic magnesium than that of total dietary magnesium.

My results in Section I (Table 2) show clearly that complete exchange before absorption does not happen, since the specific activities of the solid phase of the contents in the various sections of the tract are generally lower than those of the corresponding liquid phase from which magnesium is absorbed. Hence the figures previously obtained for availability (Field, 1959) are more relevant to the availability of $^{28}Mg$ than to that of dietary magnesium. It was not possible to ascertain whether the incomplete exchange was due to magnesium existing in chemical forms of which the stable magnesium is not readily exchangeable with $^{28}Mg$ or to some magnesium remaining in the solid phase of the ingesta out of physical contact with $^{28}Mg$ or both.

In the isotope-dilution method it is assumed that there is no exchange between exogenous and endogenous magnesium in the gastrointestinal tract, i.e. that the specific activity of the reabsorbed intestinal secretions is the same as that of the remainder of the secretions, and both Kjeriff-Jensen (1941-2) and Moore and Tyler (1955b) have questioned the validity of this assumption.

In Section I (Table 3), after intravenous administration of a single dose of $^{28}Mg$, it was found that there was a progressive decrease in the specific activity of the liquid phase from section 2 to 6 of the small intestine of sheep B, which is consistent with the selective absorption of secreted magnesium at a higher specific activity than that of exogenous

magnesium. However, since exogenous magnesium was not isolated and its radioactivity measured, limited exchange cannot be excluded.

Another major source of error in the two methods is the exchange of $^{28}Mg$ between the contents and walls of the gastro-intestinal tract, its direction depending upon their relative specific activities. There is no way of differentiating exchange from absorption after oral administration or from secretion after intravenous administration and, for this reason, both methods lead to an overestimation of availability and endogenous faecal excretion, the extent of which is unknown.

Thus from the above considerations it would appear that the assumptions inherent in the methods for the determination of availability and endogenous faecal excretion using $^{28}Mg$ are invalid and this seriously limits their quantitative usefulness. Moreover, at present, the extensive use of $^{28}Mg$ cannot be envisaged because of the high cost of the isotope.

Field et al. (1958) showed that the relationship between urinary and dietary magnesium can be used to determine availability provided the endogenous faecal excretion remains constant. There is no direct information in the literature on the variability of endogenous faecal magnesium because of the difficulty of determining it. Such information as is available on this point comes partly from studies on the fate of intravenously administered magnesium salts to dogs, man, and sheep (Mendel and Benedict, 1909; Dryere, 1936; McCance and Widdowson, 1939). As the additional magnesium is rapidly excreted in the urine, the endogenous faecal magnesium excretion is independent of the amount of

magnesium absorbed from the gut by the animal. Since endogenous faecal magnesium is secreted magnesium which has escaped reabsorption, any factor which alters intestinal secretions or reabsorption may change endogenous faecal magnesium. Because saliva is the most important source of secreted magnesium (Table 1) and because the volume of saliva secreted whilst feeding depends on the amount and coarseness of the food (Colin, 1886; Denton, 1957; Balch, 1958), it is possible that endogenous faecal excretion of magnesium, like endogenous faecal nitrogen, is linearly related to dry matter consumption. If this is so, there must be a partial regression between urinary magnesium excretion and dry matter consumption. I examined the data for urinary magnesium and dry matter consumption given by Field et al. (1958) but could find no evidence for such a relationship. Their range of dry matter consumption (790 to 1330 g/day) was practically identical with the one used in the experiments described in Section III (798 to 1330 g/day). A further possibility is that the reabsorption of secreted magnesium is dependent upon the amount of magnesium in the ingesta and hence on the dietary intake of magnesium. Studies on the absorption of magnesium with $^{28}Mg$ in Section I have shown that the reabsorption of magnesium is a very efficient process and is probably controlled by different factors from those which limit the absorption of dietary magnesium.

From the above considerations it is highly probable that endogenous faecal excretion of magnesium is a constant under normal conditions and it follows that the regression coefficient, expressed as a percentage, is the availability of dietary magnesium (Field et al., 1958). Although the

availability of dietary magnesium is generally small, the individual values (3.2, 7.4, 8.4 and 11.3 per cent) obtained for grass nuts in Section III are the smallest yet recorded for any feedingstuff.

It follows from the relationship between urinary and dietary magnesium that faecal magnesium should be linearly related to dietary magnesium with a regression coefficient equal to 1 - b, where b is the regression coefficient of urinary magnesium on dietary magnesium. This relationship is independent of the variability of endogenous faecal excretion. The relationship was found to be linear in Section III but the regression coefficient was the same for each sheep. This value, 0.88, represents an availability of 12.0 per cent, which is of the same order as the mean for the four sheep (7.6 per cent) obtained by the method based on the relationship between urinary and dietary magnesium. The failure to detect differences between individual sheep by means of the method based on faecal magnesium clearly demonstrates the difference in sensitivity in the two methods which is due entirely to the greater sampling error in the estimation of faecal magnesium. The method based on faecal magnesium, however, has one marked advantage over the other method in that it can be used for low intakes of dietary magnesium when urinary magnesium excretion is a constant representing the endogenous loss of magnesium in the urine.

In theory the negative constant terms in the regression equation of urinary on dietary magnesium is equal to the sum of the endogenous faecal excretion and the balance. Similarly, the endogenous faecal excretion is equal to the constant term in the relationship between faecal and

and dietary magnesium. In practice these terms cannot be estimated with sufficient accuracy to give reliable figures for the endogenous faecal excretion. They are subject to errors associated with the sample mean as well as the regression coefficient. The latter's contribution to the error predominates and is responsible for the large 95 per cent confidence interval found for the constant terms in Section III. For this reason it was not possible to draw any conclusions on the effect of age on endogenous faecal excretion.

To summarise the position regarding the estimation of endogenous faecal excretion of magnesium, the results show clearly that at present there is no satisfactory method for determining it. Of the three methods for the determination of availability investigated here, each of the three had a certain degree of uncertainty but it is concluded on the basis of the above discussion that the method of choice is the one based on the relationship between urinary and dietary magnesium.

Net absorption. - The net absorption value may be taken as a measure of the amount of magnesium available to the animal for growth and milk production. Rook and Balch (1958) and Rook et al. (1958) have used net absorption, expressed as a percentage of intake and designated "availability" as a measure of the utilisation of dietary magnesium. The "availability" as a comparative measure of utilisation has three disadvantages; first, it is affected by both availability and endogenous faecal excretion; secondly, it is independent of intake only if endogenous faecal magnesium is relatively small; and thirdly, it can be

determined with low accuracy because of the low availability of dietary magnesium.

In the experiments described in Section III, even when the error associated with the determination of dietary magnesium was ignored, the 95 per cent confidence limits attached to the estimates of net absorption were large relative to the estimate and equal to $\pm$ 0.137 g/day. These errors were responsible for finding, like Field et al. (1958), no detectable correlation between net absorption and either dietary or urinary magnesium.

Mechanism of magnesium absorption. - There is no direct evidence regarding the mechanism involved in the absorption of magnesium from the intestine, but certain inferences may be made from our knowledge of the transport of other cations across biological membranes. Studies on the transport of sodium have shown that differences in electric potential exist across the wall of various parts of the gastro-intestinal tract, including the rumen of the sheep (Dobson, 1955), the ileum of the rat (Curran and Solomon, 1957) and the caecum of the guinea pig (Ussing and Andersen, 1956), the intestinal contents being electro-negative with respect to the blood. Such a potential difference will almost certainly exist across the wall of the small intestine of the ruminant and will operate against the forces driving magnesium ions into the plasma from the intestinal contents.

In Section I it was found that the concentration of magnesium in the liquid phase of the ingesta increased continuously from the pyloris to the ileo-caecal junction and was always higher than that in the plasma

(Tables 2 and 3). Thus, if the absorption of magnesium was a passive process and all the magnesium in the liquid phase was in the form which undergoes transport, the main site of absorption would be near the ileo-caecal junction. The main site was found, however, to be the middle third of the small intestine, so that either one or both of the above premises are wrong. The distribution of $^{28}Mg$ in the liquid phase of the ingesta of the gastro-intestinal tract showed that the magnesium in the liquid phase existed in 2 or more chemical states, of which 1, originating mainly from the intestinal secretions, was preferentially absorbed. Thus it would appear that not all the magnesium in the liquid phase is in the chemical form, possibly ionic, which is absorbed. Passive transport would require a combined force due to concentration gradient and solvent drag in excess of that due to the electric gradient. The low efficiency of magnesium absorption by ruminants may be due either to this potential difference across the intestinal wall or to the formation of non-absorbable magnesium compounds in the intestine.

From the above considerations it follows that changes in the potential difference across the intestinal epithelium must alter the absorption of magnesium. Dobson (1959) found that high potassium concentration in the rumen increased the potential difference across the ruminal epithelium and suggested that this could interfere with the absorption of divalent cations. There is no direct evidence of the effect of potassium on magnesium absorption, although Eaton and Avampato (1952) investigated its effect at high dietary concentrations on calcium and magnesium retention by lambs. It is difficult to interpret their

results, however, since the feeding of potassium chloride to give a total of 3.2 per cent of potassium in the diet reduced the retention of both elements, whereas the feeding of hay containing the same concentration of potassium had no detectable effect.

Unlike many reports in the literature, I could find no evidence of an inter-relationship between calcium and magnesium absorption over the range of intakes investigated in Section III.

Excretion

Gastro-intestinal tract. - As expected, it was found in Section I that the main sites of secretion of magnesium from the body into the lumen of the gut were the abomasum and the first part of the small intestine. The $^{28}Mg$ found in the rumen after intravenous administration is probably derived from the saliva.

The total daily secretions of magnesium into the gastro-intestinal tract lies between 60 and 200 mg. and this is of the same order as the values found for endogenous faecal excretion by this species (Field et al., 1958; Field, 1959; MacDonald et al., 1959). Nevertheless, evidence for reabsorption was found in Section I from the study on the distribution of $^{28}Mg$ along the length of the small intestine of sheep B 10 hours after the intravenous administration of a single dose. The progressive fall observed in specific activity from the duodenum to the ileo-caecal junction is consistent with the preferential absorption of magnesium compounds of relatively high specific activities originating from the digestive secretions. One possible explanation for the conflict between

the findings may lie in the accuracy of the values obtained for endogenous faecal excretion. The comparative-balance technique (Field, 1959) and the isotope-dilution technique (MacDonald et al., 1959) have been shown to overestimate endogenous faecal excretion in Section I, while the figures obtained by the method based on the regression of urinary on dietary magnesium (Field et al., 1958) are of low accuracy because of the large error associated with the prediction of values by extrapolation from the equation. It is probable, therefore, that the values given in the literature are too high.

In the isotope-dilution method the proportion of endogenous magnesium in the ingesta is calculated by assuming that the specific activity of the gastro-intestinal secretions is equal to that of the plasma. However, this assumption appears to be invalid under my experimental conditions in Section I, since the specific activities of the walls of the sections that secrete magnesium were higher than the plasma, as was the bile. It was possible, however, to calculate from the progressive fall in the specific activity of the total ingesta from section 1 to section 6 of the small intestine of sheep B that about 75 per cent of the secreted magnesium was reabsorbed in the lower regions of the small intestine. Thus factors that interfere with the process of reabsorption may have profound effects on magnesium balance and possibly on homeostasis.

Kidney. - Magnesium absorbed in excess of the body's requirements is excreted by the kidney, though there is some doubt as to the mechanism involved. Difficulties in chemical analysis and the binding of a relatively large portion of the ion account for the relatively few studies to date of magnesium excretion. Wilson (1960) concluded with Barker et al. (1959) that "excretion of magnesium is by a filteration reabsorption mechanism in which the tubular reabsorption process is acting at or near its maximum rate and that the excretion of magnesium is partly or wholly independent of the excretion of other ions.". From this it follows that, if the tubular reabsorption rate is constant, magnesium is a threshold substance and urinary magnesium excretion is linearly related to the concentration of ultra-filterable magnesium in the plasma.

The relationship between ultra-filterable plasma magnesium and urinary magnesium has not been studied in the ruminant subject. Wilson (1960) reported values of about 2 mg/100 ml for the threshold concentration of magnesium in the plasma, but in his calculations, however, he erroneously assumed the magnesium in the plasma to be wholly filterable. When the proportion of magnesium bound to the protein is taken into account, the calculated value for the renal threshold lies between 2.5 and 4 mg/100 ml, which is inconsistent with the results obtained from the balance trials reported in Section III.

Rook et al. (1958) found a rectilinear relationship between serum or plasma magnesium concentration and the urinary magnesium concentration of cows and estimated the renal threshold for serum magnesium to be

not greater than 2.15 mg/100 ml. In contrast, no evidence for such a relationship with sheep was found in Section III, which confirms the findings of Field et al. (1958).

Since the magnesium absorbed in excess of the body's requirements is excreted by the kidney, changes in urinary magnesium excretion may be secondary to changes in intestinal absorption or changes in excretion by routes other than the kidney. Thus, if the extra-renal excretion remains constant, a correlation should exist between intestinal absorption and urinary excretion of magnesium. Because of the difficulty of determining the intestinal absorption such a relationship has not been established, but its existence is strongly implied by the finding that urinary magnesium is correlated with apparent absorption in man (McCance and Widdowson, 1942) and with dietary magnesium intake in sheep (Field et al., 1958).

Dietary magnesium depends upon the dry matter consumption and the concentration of magnesium in the dry matter of the diet. In their study of the relationship between urinary and dietary magnesium, Field et al. (1958) varied dietary magnesium by using different amounts of spring herbage of differing magnesium concentration, whereas in the current experiments of Section III dietary magnesium was changed by varying only the dry matter consumption.

The two experiments gave essentially the same results in that urinary magnesium was rectilinearly related to dietary magnesium and the regression coefficients for the individual sheep on the same experiment differed significantly. The fit of the data to a regression was

significantly better for the grass nuts than for the spring herbage, which suggests that factors existed in the different samples of spring herbage which modified slightly the relationship between urinary and dietary magnesium. These could act by changing the availability, endogenous faecal excretion or balance of magnesium.

The regression coefficients for the sheep in Section III (0.032, 0.074, 0.084 and 0.113) were generally small. The individual differences, although small, were reproducible in so far as they were the same on the two occasions they were measured, when the sheep were 4 and 5 years of age. Although the regression coefficients for the two sheep in the experiment of Field et al. (1958) were larger and the individual differences greater (0.126 and 0.263), they were not significantly different from those in the present experiment. One sheep was used in both series of experiments, and its regression coefficient was significantly lower ($P<0.05$) in the present experiment (0.032) than in the previous experiment (0.126) when the sheep was only 18 months old. This difference could be theoretically attributed to the difference in either the age of the sheep or in the diet, but further work is required to elucidate which one of the two factors is responsible.

Rook and Balch (1958) investigated the effect on the urinary magnesium excretion of dairy cows of a change from typical winter rations to spring herbage, which was cut daily from the same pasture at two stages of growth and provided much less magnesium than the winter rations. After the change to the less mature herbage, they found an immediate fall in urinary excretion and interpreted it as evidence of reduced intestinal

absorption due to the lower content and availability of the magnesium in the herbage. With the more mature spring herbage they observed a rise in urinary magnesium after an initial fall and tentatively attributed it to increased magnesium intake consequent upon a higher dry-matter consumption after the first 2 days. However, the results of my investigation in Section II show clearly that a change in diet from hay or grass nuts to spring herbage can produce a marked fall in the urinary magnesium of sheep even when the change in diet involves an increase in magnesium intake, and that the fall is followed by a progressive increase in urinary magnesium even though the intake remains constant. The complexity of the changes in magnesium metabolism associated with a change in the nature of the diet is further illustrated by the fact that the reverse dietary change to the original hay or grass nuts produced the opposite effect on urinary magnesium, namely, an immediate increase followed by a fall. The increase occurred when the dietary change involved a decreased magnesium intake and the highest urinary value was greater than those found when the hay or grass nuts were given originally. These effects were thus largely independent of the change in magnesium intake and it is conceivable that both types of effect are mediated by similar factors associated with the nature of the diets.

Since the magnesium absorbed in excess of the body's requirements is excreted by the kidney, changes in urinary magnesium excretion may be secondary to changes in intestinal absorption, or to changes in excretion by routes other than the kidney. They may also result from impaired

renal function due to a changed rate of glomerular filtration or tubular reabsorption.

Thus it is possible that the depression in urinary magnesium produced by a dietary change from dry feed to spring herbage was caused by a temporary but severe inhibition of magnesium absorption. Similarly, the increase in urinary magnesium that occurred when the diet was changed from spring herbage to hay could be interpreted as an indication of a temporary enhancement of absorption.

Although we are a long way from understanding these phenomena, they are not isolated ones, since Annison, Lewis and Lindsay (1959a, b) have described a wide range of metabolic disturbances occurring in the period immediately after a dietary change. These findings also emphasise the need to make the change-over from winter rations to spring herbage on the farm as gradual as possible.

There are other possible theoretical explanations of the changes in urinary magnesium excretion associated with changes in diet. The most obvious is that changes in the endogenous faecal excretion occur and that they influence the urinary excretion. These changes could arise from altered reabsorption of the Mg secreted into the gut, or to increased secretion possibly due to changes in the acid-base balance (Annison et al., 1959b).

A feature of the results is the individual variation in the effect of dietary changes on urinary magnesium. It was demonstrated clearly in the second experiment of Section II, in which the urinary magnesium excretion of sheep A was still depressed at the end of the period of

observation, whereas for sheep C the depression only lasted for 2 days. Further, in the third experiment of Section II, one of the four sheep failed to show a fall in urinary magnesium after the dietary change. Thus any explanation of the changes in magnesium metabolism associated with dietary change must also explain why certain animals are relatively unaffected.

Uptake of $^{28}Mg$ by the tissues

The uptake of $^{28}Mg$ by the tissues of sheep B after its intravenous administration clearly shows that there is a constant movement of magnesium into and out of the cells. Further, the rate at which exchange occurred, which is measured by the specific activity, varied in different tissues. The specific activities of the tissues and fluids were in the descending order: bile (7.1), kidney (6.4), liver (5.1), spleen (4.0), muscle (0.25), bone (0.14 to 0.05). These findings are similar to those obtained with rats by other workers. On the basis of specific activities curves, Rogers and Mahan (1959) showed that organs fall into two classes: in the liver, kidney and heart the magnesium exchanged rapidly and completely with the $^{28}Mg$ in the plasma, whereas in the brain, testes, erthrocytes and skeleton muscles there were two forms of magnesium, one rapidly and one more slowly exchangeable. MacIntyre (1959), using a similar technique, confirmed these findings and also showed that the magnesium in the bone was only slowly exchangeable and that the specific activity of the liver was always higher than that of the plasma. I observed a specific activity higher than that of the plasma for bile,

kidney and parts of the wall of the gastro-intestinal tract but not for the liver. Since magnesium in the plasma exists both free and bound, it is possible that the bound magnesium exchanges only slowly with $^{28}Mg$ and that the higher specific activity of the tissues results from exchange with ionic $^{28}Mg$ in the plasma. It is noteworthy that the rate of $^{28}Mg$ exchange in a tissue is not a measure of its magnesium reserves, since Watchorn and McCance (1937) and Blaxter, Rook and MacDonald (1954) could find no evidence of magnesium depletion in those soft tissues with high rates of exchange.

A marked variation in the specific activity from bone to bone was observed, and was greater in regions of rapid bone metabolism than in compact bone. Thus the sternal end of the rib and the epiphysis of the femur showed the greatest exchange and the femur shaft the least. A similar variation has been observed with the uptake of calcium by the same bones in cattle (Hansard, Comar and Plumlee, 1952). It is of interest that this variation in the specific activity of individual bones was in the same order as the degree of magnesium depletion in the same bones of magnesium-deficient calves (Smith, 1959d).

## SUMMARY

## SUMMARY

1. Absorption from and secretion of magnesium into the gastro-intestinal tract of sheep were investigated by following the distribution of $^{28}Mg$ in the ingesta along the tract after oral or intravenous administration of single doses of $^{28}Mg$.

Two 5-year-old wethers were given a diet of grass nuts providing 1.2 g. of magnesium daily for a period of 6 days. They were slaughtered 10 hours after receiving a single dose of $^{28}Mg$ by stomach tube (sheep A) or by intravenous injection (sheep B). $^{28}Mg$ and total magnesium were determined in the liquid and solid phases of the ingesta and in the mucosa of the various sections of the gastro-intestinal tract. Selected tissues of sheep B were also analysed.

The main site of absorption of magnesium was the middle third of the small intestine. Magnesium was secreted into the lumen of both the abomasum and the first section of the small intestine and was reabsorbed from the lower segments of the small intestine.

For both sheep the specific activities of the mucosa of those sections where absorption or secretion was not known to take place differed from that of the plasma, which was tentatively attributed to exchange of $^{28}Mg$ between the contents of the tract and the walls.

After oral administration, the ionic $^{28}Mg$ and dietary magnesium did not distribute uniformly in the gastro-intestinal tract. The specific activity of the liquid phase was generally higher than that of the

corresponding solid phase, but the difference was not constant from section to section. After intravenous administration there was no evidence of appreciable exchange between the $^{28}Mg$ in the secretions with the stable exogenous magnesium in the lumen of the gut.

There were marked variations in the specific activity between soft tissues, between bones and between parts of the same bone. The order of decreasing specific activity was bile, kidney, plasma, liver, spleen, skeleton muscle and bone.

These results are discussed in relation to the use of $^{28}Mg$ to determine endogenous faecal magnesium excretion and availability of dietary magnesium by the comparative-balance and isotope-dilution methods and it was concluded that these techniques are of doubtful value.

Samples of faeces were collected over 3 h. periods, for a total of 24 h., from 4 wethers that had been on the same dietary regime as sheep A and B for the previous 15 days. No evidence for a diurnal variation in the concentration of magnesium in faeces were obtained.

2. A study was made of the effect of dietary changes involving hay, grass nuts and spring herbage on the urinary magnesium excretion and the concentration of magnesium in the serum of adult wethers of various breeds. The samples of spring herbage were collected from fertilised leys, on one of which hypomagnesaemic tetany in cows and ewes had recently occurred.

No dietary change produced any marked alteration in the serum magnesium levels and the values remained within the normal range, even when the tetany herbage was given for 18 days.

An immediate fall in urinary magnesium excretion usually occurred after a dietary change from hay or grass nuts to spring herbage, even when the change led to increased magnesium intake. Lowest values, which occurred within 1 to 2 days of the dietary change, were followed by an increase although the magnesium intake remained constant. In some instances the excretion at the end of the period of observation reflected the difference in the magnesium content of the two rations.

The reverse dietary change produced the opposite effect on urinary magnesium, namely, an immediate increase followed by a fall. The increase occurred when the dietary change led to a decreased intake, and maximal values were observed within 1 to 3 days of the change.

The volume of urine excreted was highest 2 or 3 days after the diet had been changed from grass nuts or hay to spring herbage.

It is not known whether these changes in urinary magnesium excretion are caused by changes in absorption or in endogenous faecal magnesium excretion.

3. The results of two experiments carried out at an interval of one year are reported, each experiment comprising a series of magnesium and calcium balance trials in which each of four Cheviot wethers was given 900, 1200 or 1500 g./day of the same batch of grass nuts for 15 day periods.

For all sheep there was a highly significant rectilinear relationship between the amount of magnesium in the urine and the magnesium intake, but there were significant differences between the regression

coefficients for the individual sheep. From these equations estimates of the percentage absorption of the magnesium in grass nuts by individual sheep were obtained and ranged from 3.2 to 11.3 per cent.

The relationship between faecal magnesium output and magnesium intake differed in the two experiments; in the first it was slightly curvilinear and in the second rectilinear. These relationships gave an estimate of 12 per cent for the availability of the magnesium in grass nuts to all the sheep.

The estimation of the availability of dietary magnesium and endogenous faecal loss of magnesium by the methods based on the regression of urinary magnesium and of faecal magnesium on magnesium intake are discussed, and it is concluded that, for the former, the urine method is more sensitive and, for the latter, neither method is accurate enough to give reliable estimates.

No correlation between serum magnesium concentration and the amount of magnesium in the urine was detected but the mean value for the former was correlated with the animals' ability to absorb dietary magnesium.

For all sheep the increase in age from 4 to 5 years had no effect on the efficiency of absorption of dietary magnesium, but the concentrations of magnesium in the serum and urinary magnesium excretion were higher at 4 than at 5 years of age.

No inter-relationships between magnesium and calcium metabolism at the dietary intakes investigated could be detected. Urinary and faecal calcium excretion were correlated with intake, the former relationship was curvilinear and the latter rectilinear. From the latter an estimate of 6.4 per cent for the availability of the calcium in grass nuts was obtained.

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# APPENDIX

## APPENDIX

### Statistical analysis of the results in section III

Symbols

Main effects

Sheep S

Levels L

Experiment E

2 Factor interactions

Sheep x Levels SL

Sheep x Experiment SE

Experiment x Levels EL

3 Factor interactions

Sheep x Levels x Experiments SLE

Subscripts L and Q denote linear and quadratic component respectively.

x $0.01 < P < 0.05$ xx $0.01 > P$

In the absence of an error term the mean square of the main effects and 2 factor interactions have been tested against the 3 factor interactions.

## Analysis of variance of the urinary Mg excretion of the sheep, expressed in Mg/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 12243 | $250^{XX}$ |
| L | 2 | 5310 | $108^{XX}$ |
| E | 1 | 1411 | $28.8^{XX}$ |
| SE | 3 | 219 | |
| $SL_L$ | 3 | 518 | $10.6^{X}$ |
| $SL_Q$ | 3 | 19 | |
| $EL_L$ | 1 | 240 | |
| $EL_Q$ | 1 | 102 | |
| SLE | 6 | | |

## Analysis of variance of the faecal Mg excretion of the sheep, expressed in g/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.0199 | $6.20^{X}$ |
| L | 2 | 0.7344 | $229^{XX}$ |
| E | 1 | 0.08402 | $26.17^{XX}$ |
| SE | 3 | 0.00200 | |
| SL | 6 | 0.00395 | |
| EL | 2 | 0.01701 | $5.30^{X}$ |
| SLE | 6 | 0.00321 | |

Analysis of variance of the effect of level of feeding on the faecal Mg excretion (g/day) of the sheep in the individual experiments

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| $E_1L_L$ | 1 | 0.6670 | $208^{xx}$ |
| $E_1L_Q$ | 1 | 0.0301 | $9.37^{x}$ |
| $E_2L_L$ | 1 | 0.8001 | $249^{xx}$ |
| $E_2L_Q$ | 1 | 0.0057 | |
| SLE | 6 | 0.00321 | |

Subscripts 1 and 2 stand for the first and second experiment respectively.

Analysis of variance of the net absorption of the sheep, expressed in g/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.08544 | $12.49^{xx}$ |
| L | 2 | 0.02971 | |
| E | 1 | 0.15587 | $22.78^{xx}$ |
| SL | 6 | 0.01133 | |
| SE | 3 | 0.01524 | |
| EL | 2 | 0.02701 | |
| SLE | 6 | 0.00684 | |

Analysis of variance of the balance of Mg of the sheep, expressed in g/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.00778 | |
| L | 2 | 0.00088 | |
| E | 1 | 0.06510 | 33.08$^{xx}$ |
| SL | 6 | 0.002207 | |
| SE | 3 | 0.004693 | |
| EL | 2 | 0.006929 | |
| SLE | 6 | 0.001968 | |

Analysis of variance of the serum Mg (mg/100ml) of the sheep

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.14882 | 6.19$^{x}$ |
| L | 2 | 0.33101 | 13.76$^{xx}$ |
| E | 1 | 0.24604 | 10.23$^{x}$ |
| SL | 6 | 0.07757 | |
| SE | 3 | 0.09483 | |
| EL | 2 | 0.11648 | |
| SLE | 6 | 0.02406 | |

Analysis of variance of the urinary Ca excretion of the sheep A, B and C, expressed in mg/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 2 | 1617.72 | $6.2^{X}$ |
| $L_L$ | 1 | 26602.08 | $102^{XX}$ |
| $E_Q$ | 1 | 1667.36 | $6.38^{X}$ |
| $SL_L$ | 2 | 984.08 | |
| $SL_Q$ | 2 | 9.53 | |
| Error | 9 | 261.28 | |

Analysis of variance of faecal Ca (g/day) of the sheep

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.6186 | $6.08^{XX}$ |
| L | 2 | 20.7602 | $204.13^{XX}$ |
| E | 1 | 2.3941 | $23.54^{XX}$ |
| SL | 6 | 0.2993 | |
| SE | 3 | 0.1234 | |
| EL | 2 | 0.0936 | |
| SLE | 6 | 0.1017 | |

Analysis of variance of net Ca absorption of the sheep, expressed in g/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.61855 | 6.09$^{x}$ |
| L | 2 | 0.11973 | |
| E | 1 | 2.39402 | 23.57$^{xx}$ |
| SL | 6 | 0.2995 | |
| SE | 3 | 0.12339 | |
| EL | 2 | 0.09415 | |
| SLE | 6 | 0.10157 | |

Analysis of variance of the balance of Ca (g/day) of the sheep

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 0.5807 | 7.62$^{x}$ |
| L | 2 | 0.0714 | |
| E | 1 | 2.2632 | 29.70$^{xx}$ |
| SL | 6 | 0.3069 | |
| SE | 3 | 0.1860 | |
| EL | 2 | 0.1659 | |
| SLE | 6 | 0.0762 | |

Analysis of variance of the serum Ca of the sheep, expressed in mg/day

| Source of Variation | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| S | 3 | 6.038 | |
| L | 2 | 1.350 | |
| E | 1 | 68.694 | 49.56$^{xx}$ |
| SL | 6 | 1.968 | |
| SE | 3 | 5.624 | |
| EL | 2 | 33.561 | 24.21$^{xx}$ |
| SLE | 6 | 1.386 | |